

Therapeutic Approaches to Stroke (prevention and acute treatment)

A thesis by

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Summary

The work submitted for examination concerns several aspects of stroke patient management.

Chapter one is a general overview of the relevant literature concerning prevention of stroke both primary and secondary. The rationale for acute therapy, pathophysiology and specific treatments such as thrombolysis, anti-platelet agents, anticoagulation and novel neuroprotective agents are discussed within the introduction.

In Chapter two I examined the effects of the ACE inhibitor perindopril on blood pressure and total cerebral blood flow in hypertensive patients with recent ischaemic stroke. At present it is unclear at what stage it is safe to initiate anti-hypertensive therapy but in most cases this is delayed at least 72 hours. Patients admitted to the Acute Stroke Unit of the Western Infirmary are generally discharged either to the care of their general practitioners or to a further in-patient facility within 5 to 7 days of admission. It is therefore important to devise a risk factor intervention plan prior to discharge. Deferring decisions can result in unacceptable delays or even failure in the initiation of anti-hypertensive treatment. A total of 28 patients were recruited to the study with 24 completing the protocol. With a sample size of 24 patients we would expect to detect a difference in cerebral blood flow of 16% with 80% power.

I hypothesised that the ACE inhibitor perindopril could be instituted within 3-7 days of ischaemic stroke onset, and this treatment would be effective and safe. I used transcranial and carotid duplex Doppler ultrasound to assess any effect on cerebral blood flow. Blood pressure was effectively reduced, but there was no drug associated neurological deterioration and cerebral blood flow was unaltered. Patients were screened for underlying hypertension and following informed written consent allocated either



perindopril 4 mg or placebo for a period of 2 weeks within a double-blind, randomised, placebo-controlled study.

Blood flow was calculated from bilateral internal carotid artery Doppler ultrasound coupled to a wall tracker device. Arterial flow was calculated equal to $\pi \times \text{diameter}^2$. Doppler recordings were undertaken pre-treatment and at 2, 4, 8 and 24 hours and again at 2 weeks.

In chapter three I examined the relationship between cholesterol and outcome following stroke with surprising results. All patients admitted through the Acute Stroke Unit of the Western Infirmary had total serum cholesterol measured routinely. 1,165 patients were included in the analysis. The results of the study suggested a clear dose dependent effect of elevated cholesterol on survival following stroke. The results were, however, counter-intuitive with those patients with a significantly higher cholesterol having a better chance of survival. As the data linking cerebrovascular disease and elevated cholesterol is not wholly convincing appropriate placebo controlled intervention studies in patients with cerebrovascular disease are indicated before elderly patients should be routinely prescribed lipid lowering agents. I am currently involved in setting up such a placebo controlled study.

In chapter four I assessed the relationship between poor stroke outcome and hyperglycaemia. A number of studies have suggested a relationship between poor functional outcome and hyperglycaemia. 811 patients with computed tomography confirmed acute stroke and plasma glucose data were included in the study. The analysis was carried out retrospectively and represent consecutive admissions for which CT and immediate blood glucose data were available. Our results were consistent with the hypothesis that hyperglycaemia exerts a direct and independent effect predisposing to poor stroke outcome. These results have been confirmed by other investigators and there are a number of postulated mechanisms which have been put forward to explain this

trial of insulin therapy to correct hyperglycaemia versus standard observation in patients with acute stroke.

Chapters five, six and seven were phase II placebo controlled trials of novel neuroprotective compounds currently being evaluated as treatment for acute stroke. The studies were not powered to demonstrate efficacy but rather to evaluate tolerability, safety and clinical pharmacology prior to phase III studies.

In chapter five we evaluated the safety and tolerability of GV150526 (a glycine receptor antagonist) in patients with acute stroke. This drug was found to be extremely well tolerated when compared with other neuroprotective agents and the results suggest that putative neuroprotective concentrations can be achieved in patients with good tolerability. We observed a hitherto unrecognised effect on liver function. These observations lead to further toxicology studies. The results of the study and pharmacokinetic analysis have been utilised in the design of a phase III clinical efficacy study.

Chapter six evaluated the safety and tolerability of remacemide hydrochloride, a neuroprotective NMDA antagonist with anticonvulsant activity. While putative neuroprotective levels were reached pharmacokinetics suggested that the active metabolite took some time to attain “therapeutic” levels and that rises in metabolite were associated with poor tolerability. Nausea, vomiting and CNS side effects such as hallucinations and agitation were commonly reported at the higher doses. In part due to the poor clinical tolerability but also because of the pharmacokinetic profile remacemide has not gone on to phase III evaluation in stroke patients, although it continues to be developed as an anticonvulsant.

In chapter seven we evaluated the tolerability of bolus single doses of aptiganel. Having predicted an appropriate infusion rate from the initial bolus pharmacokinetic data we assessed the effect of bolus followed by an appropriate infusion to maintain plasma levels at a steady state. Aptiganel is known to cause dramatic CNS effects and occasional hypertensive responses. The original dose selected for bolus and infusion was not well tolerated due to the frequency of CNS and significant hypertensive effects. A second dose and infusion regimen was then investigated. While there were CNS and blood pressure adverse events reported the results were deemed encouraging enough to proceed to a phase III efficacy study.

All studies within the thesis were approved by the West Ethics Committee. Written informed consent was obtained from all patients entering into chapter two. In chapters five, six and seven informed consent was obtained wherever possible although informed assent was also considered acceptable in patients with speech or comprehension difficulties. The studies in chapter three and four were epidemiological and consent was not required.

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Declaration

The work described in this thesis was carried out while I was employed as a Research Fellow in the Department of Medicine and Therapeutics at the Western Infirmary. Dr Chris Weir was first author in the hyperglycaemia study and assisted in the statistical analysis of the cholesterol study. Dr Donald Grosset devised the original protocol for the study of perindopril. Dr Keith Muir was initially involved in the medical supervision of the remacemide study. The writing of the thesis was otherwise entirely my own work.

Introduction

This chapter will review stroke as a medical problem, discuss risk factors and their management, and introduce the background to neuroprotective interventions.

Stroke diagnosis, prevention and management of hypertension

1.01 Diagnosis

Stroke is responsible for 12% of all deaths in England and Wales¹ with 2339 males and 2720 females dying in the UK in 1992.² It is the third greatest cause of mortality in Britain, superseded only by ischaemic heart disease and all cancers combined and has a profound social and economic effect not only on individuals who become disabled, but on society as a whole. The prognosis of patients with stroke is worse than for many forms of cancer, with half of all patients dead or dependent on carers after one year. The outcome is even more bleak for patients with severe stroke with 96% patients dead or dependent following a total anterior circulatory syndrome (TACS).³ Despite recent encouraging results with rt-PA, there is presently no widely applicable treatment for the majority of patients with acute ischaemic stroke.⁴ Effective means of secondary prevention: i.e. aspirin; anticoagulation in patients with atrial fibrillation; and carotid endarterectomy in patients with high grade carotid stenosis are now well established and are proven to reduce stroke recurrence.

The process of stroke diagnosis begins with recognition that a focal neurological deficit of sudden onset has arisen. The main differential diagnoses include epilepsy and migraine; these are distinguished from stroke mainly on the basis of the clinical history. Rarer possibilities include hypoglycaemia, intracerebral tumour, demyelination, syncope, subdural haematoma, malignant hypertension and giant intracerebral aneurysm.⁵ The diagnosis of a vascular event is therefore largely based on the clinical history and examination findings. The importance of reaching an accurate diagnosis in the cases with good recovery is

paramount, to enable appropriate secondary preventive measures to be planned. It is no longer acceptable clinical practice to diagnose a cerebrovascular event without defining this further, and identifying the likely aetiology.⁶ The first distinction that must be made is between intracerebral haemorrhage and infarction. Despite the promulgation of clinical scoring tools to distinguish between the two,⁷⁻⁹ scoring systems remain equivocal in a substantial proportion of patients and may be dangerously misleading in others.¹⁰ Since anti-thrombotic and anticoagulant treatments are increasingly recognised for secondary prevention of ischaemic stroke, it is essential to exclude haemorrhage in most cases.¹¹ Computed tomography (CT) scanning is the most reliable clinical tool currently available⁶ but should not be delayed beyond 2 weeks after the cerebrovascular event, since the appearances of a cerebral haemorrhage gradually alter and may become indistinguishable from infarction. Magnetic resonance imaging (MRI) may be a better option when a prolonged delay in investigation has occurred, but is more expensive and not freely available in all centres.

In ischaemic stroke the second element of diagnosis is to establish the vascular territory which has been affected.¹² Carotid territory stroke should be distinguished from ischaemia in the posterior circulation, since patients with symptomatic carotid disease may benefit from carotid endarterectomy. Patients with infarctions in the watershed regions between major vessels (posterior, middle and anterior cerebral arteries) may have suffered systemic hypotension with or without occlusive disease in a proximal artery.¹³ These patients should be investigated for a precipitating cause e.g. silent myocardial infarction or arrhythmias.

The third element of diagnosis is to establish the pathophysiological processes which have eventually lead to cerebral ischaemia and correct as many of these modifiable risk factors as possible.

Hypertension predisposes to both large and small vessel disease. Hypertension is the major risk factor for small vessel cerebrovascular disease, which is probably neither influenced by hyperlipidaemia nor amenable to surgical intervention. Small vessel disease predominantly leads to so-called 'lacunar' strokes. These may be recognised clinically as causing pure motor or pure sensory involvement of at least two adjacent elements of the face, arm and leg, or by the 'dysarthria clumsy hand syndrome' or an ataxic hemiparesis.

Cortical strokes, i.e. those involving higher cerebral dysfunction (neglect or dysphasia) often accompanied by hemiparesis and / or hemianopia can be attributed to large vessel or embolic disease. The practical distinction that must be made is between those strokes caused by carotid stenosis, causing occlusive or embolic symptoms, and those due to cardioembolic disease. Patients with significant (>70%) stenosis should be considered for carotid endarterectomy, as this is now an established and effective secondary preventive measure (figure 1.01.1). Unfortunately both may coexist making a firm diagnosis impossible.

Carotid disease is best diagnosed and quantified by colour flow duplex ultrasound (figures 1.01.2 and 1.01.3). The presence or absence of carotid bruits should be disregarded as they are not reliable indicators of underlying haemodynamic compromise. Cardiac investigations seeking embolic sources or cortical stroke in particular should be considered where clinical features suggest atrial fibrillation, a structural cardiac abnormality or recent myocardial ischaemia. Echocardiography should be reserved for patients with clinical or electrocardiographic evidence of a cardiac abnormality and for patients with no other risk factor for stroke. Patients with recurrent episodes of ischaemia in different cerebrovascular territories may have a central source of embolism, e.g. arising from the heart or arch of the aorta, and should be investigated with trans-thoracic and even trans-oesophageal echocardiography.

The secondary prevention issues which arise primarily concern three approaches: anticoagulation for atrial fibrillation or presumed cardioembolic stroke; carotid endarterectomy for symptomatic ipsilateral carotid stenosis of 70% or more (but excluding carotid occlusion); and antihypertensive treatment.

Primary prevention of stroke by antihypertensive treatment is well established.¹⁴ Secondary prevention is likely to achieve even greater benefits since the absolute risk of stroke is substantially higher in such patients. Blood pressure management early after acute stroke is controversial. These issues will be discussed in detail.

1.02 Stroke Risk And Hypertension

The association between hypertension and the incidence of cerebrovascular disease and in particular acute stroke is now well established. A blood pressure reduction of 10-12/5-6 mmHg confers a 38% reduction in stroke incidence.¹⁴ Unfortunately evidence suggesting that treating hypertension is an effective secondary preventative measure in patients following stroke or TIA is still scanty but the focus of current research interest.

The epidemiological evidence isolating hypertension as an independent risk factor for the primary incidence of both stroke and coronary heart disease came from nine prospective non-randomised observational studies.¹⁵⁻²³ These studies involved almost 420 000 patients followed up, on average, for a period of 10 years. Thus, a 5 mmHg reduction in diastolic BP anywhere in the range of BP is associated with a 30% reduction in stroke incidence while a 10 mmHg BP reduces this by 50%. Furthermore the relationship between BP and incidence of stroke is approximately log linear,²⁴ with no apparent 'threshold' level of blood pressure below which the association is lost (figure 1.02.1).

Collins and MacMahon have suggested the initial strength of this association may have been significantly underestimated as a result of sampling errors. Many of these trials took blood pressure measurements as a baseline value prior to entry into the study. These values took no account of random fluctuations in the blood pressure and errors in blood pressure estimation itself. The result of this 'regression dilution bias'²⁵ is usually an underestimation of the strength of association between two variables, in this case blood pressure and disease incidence.²⁴ Data from the Framingham study²¹ suggest correction for this form of error increases the gradient of association between blood pressure and stroke by 60%.

The relationship between BP and the secondary incidence of stroke has not as yet been fully established. The UK TIA trial suggested a continuous direct association between BP and stroke recurrence across the range of blood pressures similar to the primary incidence data.²⁶ Again there was no evidence of a threshold level of BP below which the relationship was lost (figure 1.02.2). Other investigators have postulated a J-shaped relationship between BP and stroke recurrence rates, suggesting diastolic BP's < 80 mmHg are associated with an increased risk of stroke recurrence²⁷ (figure 1.02.3) A similar curve describes the relationship between BP and recurrent myocardial infarction.²⁸ One possible explanation for the J-shaped curve is that patients with pathologically low blood pressures due to poor LV function have an increased risk of both stroke and myocardial infarction²⁹.

1.03 Rationale for treating hypertension as a means of stroke prevention

Hypertension is an important precursor of both cerebrovascular disease and ischaemic heart disease. The incidence of cerebral infarction and haemorrhage are directly related to underlying hypertension while the relationship with subarachnoid haemorrhage is more complex with conflicting data from epidemiological, laboratory and clinical studies.³⁰ Although stroke is a heterogeneous condition with many different causes and pathologies hypertension accelerates and contributes to the development of many of these. Coronary heart disease, large vessel atheroma formation, aneurysmal dilatation intracerebral haemorrhage and arteriosclerosis of small vessels are examples.³¹ Large blood vessel changes induced by hypertension include hypertrophy of smooth muscle and elastic fibres, leading eventually to fibrous replacement.³² The vessel walls become increasingly thickened, rigid, elongated and tortuous with dilated lumina. Small vessel lumina are reduced due to intimal thickening and undergo hyaline arteriosclerosis, an age-dependent process accelerated by underlying hypertension. Cerebral arteries develop lipohyalinosis³³ and miliary aneurysms which can rupture leading to primary intracerebral haemorrhage.³⁴ This most frequently affects the perforating arteries of the basal ganglia, cerebellum and periventricular white matter.

Severe hypertension also leads to thrombotic occlusion of arterioles. The overall effect of these changes increases cerebrovascular resistance and therefore reduces cerebral perfusion.³²

Patients with hypertension also have a reduced ability to maintain cerebral perfusion in the face of fluctuating systemic blood pressures. Cerebral autoregulation is a dynamic homeostatic system which maintains cerebral perfusion at a constant level despite changes in systemic blood pressure by altering the cerebral vascular resistance. In a normotensive individual the lower limit of autoregulation is 60-70 mmHg (mean arterial BP). Below this level, but between pressure levels of 40-60 mmHg the brain can compensate by increasing its extraction of O₂. When BP falls further brain hypoxia develops and cell death will occur.³⁵ In hypertensive patients the autoregulation thresholds are set at an abnormally high level, i.e. 85 mmHg,³⁶ thus predisposing the individual to hypoperfusion of brain tissue should blood pressure fall dramatically.³⁷ It is likely but unproven that the extent of autoregulatory resetting depends on the severity of the hypertension. Autoregulatory resetting could theoretically increase the likelihood of watershed infarction in the event of a hypotensive episode and cases of neurological deterioration have been documented following sudden lowering of BP to 'normal' values. Recent research in animals has shown that correction of blood pressure can lead to correction of the autoregulation set-point.^{38,39} Similar studies in humans have however as yet failed to demonstrate significant resetting of the autoregulation threshold in either normotensive or hypertensive subjects.⁴⁰ Hypertension consequently contributes to the development of a myriad of pathological mechanisms all of which make cerebral infarction or haemorrhage more likely.

It is logical to assume that control of hypertension may reduce the future risk of stroke by halting or reversing these mechanisms.

1.04 Benefits of primary prevention

Thus far 17 randomised placebo-controlled trials of antihypertensives have been performed. involving 47,000 patients known to be hypertensive but without symptomatic cerebrovascular disease.²⁴ A total of 1360 strokes were detected over a follow-up period of, on average, 5 years. The reductions in stroke risk associated with a particular drop in BP were even more impressive than extrapolation of the epidemiological evidence might have predicted. A reduction in diastolic BP of 5mmHg was associated with a 38% reduced risk of stroke (figure 1.04.1): while epidemiological evidence from the the Framingham study suggested only a 30% reduction was likely. The results of these trials also suggested that most, if not all, of the benefits associated with blood pressure reduction are apparent within 2-3 years of treatment commencing.

The proportional reductions in stroke risk were similar for trials using higher or lower blood pressures as inclusion criteria, i.e. there appears to be no additional benefit from lowering BP in patients with higher baseline values. If these group risk reductions are applicable to individual patients, it could be concluded that maximal benefit in terms of numbers of strokes prevented per patient treated would be observed in those patients at higher risk of stroke. Middle-aged hypertensives have an annual incidence of stroke of approximately 0.5% ¹⁴ whereas older hypertensives have a greater risk of 1.5%.⁴¹ If treated for a period of 10 years 50 low-risk patients compared to 17 high-risk would be required to prevent one stroke.

Recent studies emphasizing the importance of treating hypertension in the elderly population have lead to a new awareness of the potential benefit from active antihypertensive intervention in this group of patients. The SHEP investigators⁴² demonstrated reduced total mortality, cardiovascular mortality and morbidity and stroke in patients aged over 60 years of

age with isolated systolic hypertension. The relative reduction in stroke risk was 36%, demonstrating that, at least in the elderly, systolic blood pressure has prognostic implications independent of diastolic values (figure 1.04.2).

1.05 Benefits of antihypertensive therapy as secondary prevention of stroke

It might be postulated that the greatest benefit from antihypertensive treatment would be derived from patients who have suffered either a TIA or stroke, where the risk of recurrence is at least 5% per annum.⁴³ While it is hoped that the benefits of treating hypertension observed in large clinical trials in patients asymptomatic for cerebrovascular disease might be mirrored in this group of patients, as yet no definitive study has been conducted.

Retrospective observational analysis of mortality and stroke recurrence in Rochester, Minnesota, USA, failed to reveal an association between blood pressure and stroke recurrence rates, throughout a 30-year follow-up period in a total of 1600 patients.⁴⁴ In a more recent study in patients following a transient ischaemic attack a direct continuous association between blood pressure and the secondary incidence of stroke was observed.⁴⁵ The epidemiological evidence is therefore not as conclusive or complete as the data linking the primary incidence of stroke and hypertension.

Secondary prevention data are available from a mere two trials including a total of only 549 hypertensive patients.¹⁴ Two further studies have been carried out treating normotensives following a TIA with atenolol. These trials recruited 2193 patients but only modest reductions in blood pressure were demonstrated (2-3 mmHg diastolic).^{45,46} Patient numbers were, as a result, inadequate to demonstrate the epidemiologically predicted benefits in lowering stroke risk (20%). If all four studies data are pooled together, the results suggest a benefit in stroke

risk reduction of 19%, but with wide confidence intervals (95% CI: -49%, +11%) (figure 1.05.1). Future studies in hypertensive patients where BP is more conclusively lowered may reveal a greater reduction in the stroke incidence.

A large placebo-controlled trial (PROGRESS) is currently under way assessing the efficacy of a thiazide diuretic and/or the angiotensin converting enzyme (ACE) inhibitor perindopril as secondary prevention for stroke in mildly hypertensive and normotensive individuals.^{47,48} It is hoped that by recruiting 6000 patients and following them up for a prolonged period (5 years) the trial will be sufficiently powered to demonstrate the benefit of antihypertensive therapy in patients with cerebrovascular disease.

While trial and epidemiological evidence suggests the greatest benefits to individuals are gained in those patients at highest risk, the potential for reducing the overall population stroke incidence in younger mild hypertensives and even normotensives is, nevertheless, substantial as numerically they make up most of the population. While it would be inappropriate to intervene pharmacologically with all these individuals, public health measures to reduce blood pressure, e.g. by promoting exercise, reducing obesity, or lowering salt intake, could conceivably be hugely effective in reducing the future stroke risk of millions.

As yet, no specific group of antihypertensive agents have been identified as more effective than any other. Comparative studies of thiazide diuretics and beta-blockers have been performed in four trials involving 23,000 hypertensive patients.⁴⁹ A statistically insignificant 13% greater reduction in stroke risk was noted in the thiazide group. As yet, a comparative trial with sufficient statistical power to elucidate the effects of different antihypertensive medications on mortality and stroke recurrence has not been performed.

1.06 Management of hypertension in acute stroke

Assessment of hypertension after an acute stroke is complicated by physiological changes which influence the level of blood pressure itself and the effects of blood pressure lowering therapy on patients with cerebral ischaemia. First, cerebral autoregulation is impaired after stroke.⁵⁰ Cerebral perfusion is proportional to systemic BP and reduction of BP acutely may impair perfusion to the ischaemic penumbra. The penumbra is defined as an area of brain tissue where electrical activity is lost but the potential to recover electrical activity and contribute to a useful functional recovery still exists.^{51,52} Any reduction in blood flow to such a critically perfused area could mean the difference between irreversible infarction and the resolution of symptoms and recovery.

Many case reports suggest lowering of BP acutely in patients with ischaemic stroke leads to a worsening of clinical outcome,⁵³ and evidence from therapeutic trials of the calcium antagonist, nimodipine, supports this view. Early BP reduction in this study was associated with an adverse functional outcome⁵⁴ (figure 1.06.1). Similar findings were noted in a small pilot study assessing the ion channel blocker lifarizine: in this case, a significant drop in the blood pressure of elderly females receiving active drug was associated with a poorer functional outcome.⁵⁵

Blood pressure reduction may be specifically contraindicated in the presence of significant carotid and/or vertebral stenosis. The most obvious example would be a patient with wide-spread vascular disease who has bilateral carotid stenosis, perhaps having progressed to unilateral occlusion, and severe systolic hypertension. Just as BP reduction may impair renal function in the presence of bilateral renal artery stenosis, the risk of ischaemic stroke may be increased by reduction of BP.¹³ There little scientific evidence to support this theory but a

significant number of hypertensive patients have antihypertensive therapy withheld because of these concerns.

Dose dependent increases in blood pressure are seen with the NMDA antagonist aptiganel, with rises of mean arterial blood pressure up to 30mmHg. This has not been shown to affect total cerebral blood flow but MCA velocity is increased.⁵⁶ These blood pressure effects have now been reported in stroke patients (chapter 7). It is conceivable that rises in systemic blood pressure could lead to exacerbation of cerebral oedema or even haemorrhagic transformation of infarction, but it is equally possible that moderate rises in BP are beneficial to outcome by increasing local perfusion and improving blood flow. Deliberate attempts are made to increase BP and reduce delayed cerebral ischemia after subarachnoid haemorrhage by a combination of hypervolaemia, hypertensive agents, and hyperventilation.

Drugs lowering BP are therefore likely to have any neuroprotective effect obscured by an adverse BP lowering effect, while the effect of modest rises in blood pressure is as yet unknown and requires further research.

There is no doubt that in cases of severe hypertension especially if associated with papilloedema, retinal haemorrhages and soft exudates (accelerated or malignant phase hypertension) blood pressure should be reduced. Severe hypertension is characterised not only by cerebral autoregulatory failure but also by fits and focal neurological damage. At present few clinicians would intervene to lower blood pressure by pharmacological means unless blood pressure was sustained for several hours at over 120 mmHg diastolic and or 220 mmHg systolic, or in patients with other clinical features of accelerated phase hypertension.⁵⁷ There are some circumstances when cautious blood pressure reduction should be considered at a lower threshold. These include patients with other cardiovascular events such as aortic aneurysm, acute myocardial infarction, heart failure, primary

intracerebral haemorrhage or haemorrhage due to arterial dissection or aneurysmal rupture.^{6,57}

Approaches to the management of raised blood pressure in acute stroke are complicated by the well documented rise in blood pressure which commonly occurs in patients within the first 48-72h of admission to hospital after stroke.^{58,59} This rise and subsequent spontaneous fall appears to be related to the physical and psychosocial stresses of admission to hospital and not to the acute ictal event⁵⁹ (figure 1.06.1). Short-term blood pressure elevation may be associated with bladder distension, and pain. Systematic studies have revealed evidence of activation of the renin-angiotensin system and sympathetic nervous system in patients with acute stroke. This activation is likely to contribute to the observed transient elevation of blood pressure. The rise in blood pressure occurs in those who were previously normotensive as well as in patients with a history of hypertension. In the case of the latter they start from a higher baseline, rise to higher levels and subsequently fall to their hypertensive baseline. While it is widely believed that this transient rise in blood pressure is not of pathological significance, it must be acknowledged that this conclusion is not based on any formal randomised observation or intervention study. It is therefore important to recognise that many stroke patients with hypertensive BP readings on admission will spontaneously normalise their BP within the next 5-7 days. Decisions about long-term treatment of mild to moderate hypertension in stroke patients should therefore be based on blood pressure recordings weeks or months after the stroke and on other considerations such as evidence of target organ damage (electrocardiographic and echocardiographic left ventricular hypertrophy, renal impairment, proteinuria, etc.). There is probably more justification in treating hypertension in association with lacunar infarction, particularly where other cardioembolic or associated risk factors are absent.

It may be justifiable to manage hypertensive patients with intracerebral haemorrhage more aggressively as this may contribute to continued bleeding and further rises in intracranial pressure. These patients tend to show a more marked acute hypertensive reaction after stroke with a more protracted period of elevated blood pressure.⁵⁹

As discussed above, it is only on infrequent occasions that blood pressure requires urgent reduction in patients with acute stroke. In our Acute Stroke Unit with over 700 admissions each year, such treatment is indicated less than once a month. Unfortunately, there are few if any adequately controlled studies to guide the choice of drug therapy in acute stroke. Extrapolation of experience from other hypertensive emergencies and urgencies is probably not justified in view of the particular haemodynamic circumstances of acute stroke discussed above.

The choice of drugs and formulations varies widely between countries. If patients are unconscious or have swallowing difficulties, an intravenous preparation will usually be required further limiting the choice. Ideally blood pressure should be reduced in a predictable and controlled manner to intermediate levels of 160-170/90-110 mmHg for the first 5-7 days. Uncontrolled, excessive falls or prolonged reductions in pressure should be avoided.⁶ Intravenous infusion of sodium nitroprusside or nitrates in the short-term offers the optimal potential for control of the fall in blood pressure with rapid reversal of the effect should that be necessary. Diazoxide or hydralazine intravenously were formerly used but may cause excessive falls in blood pressure or marked reflex tachycardia, while intravenous clonidine or labetalol have been used in some North American and European clinics.

In the absence of sound evidence from clinical trials the choice of drugs is largely empirical and likely to be influenced by other associated medical conditions. Amongst the newer drugs.

angiotensin-converting enzyme (ACE) inhibitors deserve formal evaluation in acute stroke not least because preliminary evidence suggests that unlike many older antihypertensive agents ACE inhibitors do not acutely reduce cerebral blood flow in ischaemic stroke.⁶⁰ ACE inhibitors have been shown to reduce the incidence of recurrent myocardial infarction.^{61,62} Studies in animals suggest possible additional benefits by reducing atheromatous plaques in both carotids and other vessels.⁶³⁻⁶⁵ The American Heart Association guidelines⁶ suggest that the ACE inhibitors or calcium antagonists can be used but warn of precipitous falls after 'sublingual' calcium antagonists.

Cerebrovascular disease remains a major complication of inadequately treated hypertension. Physicians who manage hypertensive patients should acknowledge the challenges which face them. The optimal management of BP is not known, either immediately following stroke, or in normotensive survivors of stroke. Primary prevention could also be improved by greater attention on the part of cardiovascular physicians to anticoagulation in patients with atrial fibrillation since few other groups of doctors are in a position to provide advice and follow-up on the scale which is required. Further research is required to establish the optimal treatment of the hypertensive patient with cerebrovascular disease.

1.07 Rationale for acute intervention in stroke

In the United States the American Heart Association has published guidelines on the management of stroke patients with particular regard to blood pressure control, the need for CT scanning, and the indications for carotid endarterectomy as secondary prevention. In the UK these guidelines are not widely practised and a recently conducted survey of consultant opinion in the UK demonstrated widely differing views on management, even on the necessity for CT scanning.⁶⁶ This reflects the lack of concrete trial evidence supporting any form of interventional therapy and a degree of apathy within the medical profession. There has however been an explosion of knowledge regarding the pathophysiology of cerebral ischaemia and infarction and a rigorous effort is in progress to develop drugs which may preserve brain tissue by interfering with the processes that give rise to and exacerbate ischaemia.

Physicians treating stroke patients have taken heart from the results of the National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group (NINDS) study of rapid thrombolysis of patients with CT confirmed stroke. The results show a reduction in mortality and morbidity in patients receiving thrombolysis within 3 hours of onset of symptoms.⁴ This is the first good evidence that an as yet undefined time window of opportunity exists for the treatment of stroke not only in animal experimental models, but also in humans. To decide on a strategy for the development of any potential new treatment we must first consider the processes that lead to stroke, and the pathophysiological changes that take place within ischaemic brain tissue and that contribute to the eventual extent of the infarct.

1.08 Pathophysiology of ischaemic stroke and its influence on treatment strategies

Stroke is a heterogeneous condition most commonly precipitated by large or small vessel atherothrombosis; it may also result as a consequence of embolism from the heart, the arch of the aorta or carotid vessels; and a number of other rarer causes e.g. trauma leading to vertebral artery dissection, haematological problems such as hypercoagulation or hyperviscosity states, and neurovascular abnormalities e.g. Moya Moya disease. A further 15%-20% of strokes are due to primary intracerebral or subarachnoid haemorrhage. Since a variety of different insults can precipitate cerebral ischaemia, it is unlikely that any one mode of treatment will be of benefit to all patients.

The brain is dependent on a rich supply of both oxygen and glucose. When blood flow to the brain ceases abruptly i.e. following cardiac arrest, unconsciousness and irreversible neuronal damage rapidly ensue and infarction develops if adequate perfusion is not rapidly reinstated.⁶⁷ Evidence from animal models of stroke suggest that following an ischemic insult neurological damage spreads out circumferentially from the central core of the infarct.⁶⁸ If ischaemia is present for over an hour the volume of infarction gradually enlarges to its maximal size over a period of 3-4 hours in rodents⁶⁹ and 6-8 hours in non-human primates⁷⁰ and an as yet undetermined time in humans.

Experiments in baboons investigating the effects of ischaemia on neurone membrane potentials indicate a critical blood flow threshold value ≤ 15 mL /100g per minute associated with electrical silence, despite normal membrane potentials and almost normal levels of ATP.⁷¹

At flow rates ≤ 10 mL / 100 mg per min total energy failure occurs and ATP becomes depleted resulting in the breakdown of normal ionic homeostasis. Potassium efflux and sodium influx follow associated with dramatic increases in the intracellular calcium concentration.⁷² The levels of flow between these two thresholds offer conditions in which tissue can either become irreversibly ischaemic or recover. It is this so-called 'penumbral' area which it is hoped will be amenable to therapeutic salvage.

In man positron emission tomography (PET) techniques have demonstrated reversible ischaemia up to 48 hours after stroke but it is not known whether this represents the true window of opportunity for treatment.⁷³ This new technology now means it is possible to study changes in local cerebral metabolism and blood flow in humans. PET scanning gives details on the relative extraction fractions of oxygen, the quantity of oxygen and glucose metabolised, and the regional perfusion of ischaemic areas. Dense ischaemia is associated with irreversibly reduced metabolism of both oxygen and glucose. Results from these patient studies have confirmed what was already believed from the above primate studies. Regional cerebral blood flow of below 12mL/100g /min or regional metabolic rates for oxygen below 65 $\mu\text{mol}/100\text{gr}/\text{min}$ were associated with irreversible necrosis.⁷⁴ When perfusion was less drastically reduced i.e. in those penumbral areas surrounding the densely ischaemic core, cerebral metabolism of glucose and oxygen was still maintained by a relative increase in oxygen extraction. The brain can therefore compensate for small reductions in perfusion by increasing its extraction of nutrients.

Animal models of stroke

Before any potential treatment can be assessed within clinical studies in man a sound rationale for its use must be demonstrated. This inevitably involves preclinical testing within cell culture or animal models of stroke. Cell culture can be used to screen a large number of potentially neuroprotective compounds. A variety of neurotoxic insults has been used including hypoxia, excitatory amino acid agonists, and activators of sodium channels.⁷⁵⁻⁷⁷ Since stroke is a neurological manifestation of a vascular disorder, reliable animal models of the vascular causes of stroke have been developed.

There are basically two in vivo animal models of cerebral ischaemia. Models of global ischaemia are more representative of conditions following cardiac arrest and prolonged anoxia whereas models of focal ischaemia, i.e. the middle cerebral artery occlusion (MCAo) model, mimic the effects of a large vessel occlusion in humans.⁷⁸ Most studies have been conducted using small rodents i.e. rat or gerbil as these animals are inexpensive to maintain. Ischaemia can be induced artificially using a number of techniques to occlude large vessels like the MCA i.e. intraluminal filament insertion, electrocoagulation, or extraluminal compression. Embolic stroke can be simulated by injecting polystyrene microspheres into the cerebral circulation.⁷⁶ In animal models of stroke very low flow caused by occlusion of major cerebral vessels leads to immediate weakness of limbs and infarction develops within 5-10 minutes if blood flow is not re-established.⁷⁹ Less dramatic reductions in blood flow can be induced by occluding the middle cerebral artery of animals (MCAo model). Infarction develops over 3-4 hours in rodents⁶⁹ and 6-8 hours in non-human primates⁸⁰ and an as yet undefined period in humans.

A large number of potentially neuroprotective as well as thrombolytic compounds have been evaluated using these models. Unfortunately due to the practical and ethical issues in conducting such experiments in higher primates, only limited data from old studies are available on species more closely related to man. Results of studies with thrombolytics and neuroprotective agents in rodent models are encouraging with substantial reductions in infarct volume when drug is administered before, during or even after the vascular insult.⁸¹

Results in human trials have failed to demonstrated efficacy for treatment other than thrombolytics. There are a number of reasons why this may be so: the anatomical difference between rodent brain and man, the predominant use of relatively young healthy animals unrepresentative of human patients with stroke, and the careful control of oxygen saturation, blood pressure and temperature. Furthermore, pre-clinical investigators can, to a large extent, control the ratio of densely ischaemic tissue / ischaemic but viable tissue. The methods used to generate cerebral ischaemia within these models are therefore more controlled than in the clinical setting and may overestimate the potential benefit of therapy.

1.09 Mechanisms of neurotoxicity

Recent interest has focused on the potential role of the N-methyl-D-aspartate (NMDA) receptor and excitatory amino acids (EAA) in the development of cerebral infarction. While the reduction in oxygen and nutrient supply to the brain provides the initial insult to ischaemic neurones, tissue damage is exacerbated by a complex cascade of receptor mediated events. In normal physiological conditions these receptors mediate essential neurological functions such as cognition, memory, movement and sensation. Varying insults may give rise to the

same 'common pathway' of neurological damage i.e. hypoglycaemia, AIDS dementia complex, motor neurone disease and possibly Alzheimers disease.⁸²

Excitatory amino acids are thought to have a crucial role in the development of neurological damage once cerebral perfusion is reduced to a level where metabolic activity is compromised but may potentially still recover.⁷⁸ Recovery of perfusion through collateral circulation may lead to a resolution of neurological function, but further neurological damage may occur due to the subsequent build up of EAAs.^{69,73,80} The best evidence of this comes from animal models of stroke where antagonism of the NMDA receptor reduces infarction volume by as much as 50%.

Glutamate is the most abundant EAA in the human brain and is stored in presynaptic vesicles. Normally its release from presynaptic terminals is mediated by exocytosis and diffusion across the synaptic cleft where it exerts an excitatory effect on post-synaptic neurones. Glutamate is physiologically cleared from the extracellular space and deposited in astrocytes and presynaptic neurones by energy dependent uptake systems. It is then converted to glutamine through a further energy requiring enzyme system. These mechanisms are dependent on the normal physiological gradient of sodium and potassium across the cell membrane. During ischaemia, the resultant energy loss leads to these transport and conversion systems failing or even acting in reverse.⁸³⁻⁸⁷ Amino acids are also produced by injury to neighbouring cells initiating a further potent positive feedback loop of increasing excitotoxicity and rising glutamate levels. Activation of EAA receptors lead to influxes of sodium, chloride and water which lead to cellular swelling but it is the entry of extracellular calcium and mobilisation of intracellular calcium which lead ultimately to neuronal death.

There are two main groups of glutamate receptors: ionotropic receptors are coupled directly to membrane ion channels controlling calcium or sodium influx and metabotropic receptors

are linked to intracellular phosphatidyl inositol enzyme systems. Ionotropic receptors can be split into three groups according to their selective agonists: N- methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-isoxazolepropionate (AMPA) and kainate.⁸¹ Thus far, antagonists of the two former ionotropic receptors have been developed and assessed as potential neuroprotectives in animal models of stroke while little is currently known regarding the detail of metabotropic receptor function. A threshold relationship between excess glutamate release and cerebral blood flow exists whereby elevations in glutamate are triggered by flow rates below 20 mL per 100g of brain tissue per minute.⁸⁸ This is a similar flow rate to the threshold associated with ischaemia i.e. 17 mLs / 100g /min⁸⁹ and is substantially higher than flow rates leading to hypoxic energy failure and cell death (10mL / 100g /min).⁷² This suggests a grey zone between the onset of the increase in EAA's and irreversible neurotoxicity. It is hoped that neuroprotective drug therapy could salvage part of this 'penumbral' brain tissue by antagonising EAA's and their effects.

The role of calcium in neurotoxicity

There are two subsets of voltage sensitive calcium channels of importance in mediating excitotoxic damage. Activation of L-type receptors leads to a post-synaptic calcium leak that may be blocked by conventional calcium blocking drugs, such as nimodipine.⁹⁰ Drugs in this group are neuroprotective both in cell culture and in in-vivo animal models of stroke.⁹¹ N-type calcium channels are mainly found in neuronal tissue and control the release of a number of synaptically stored neurotransmitters, including the excitatory amino acids glutamate and aspartate. Opening of these channels and the resultant calcium influx add to the cycle of excitotoxic amino acid release in another positive feedback mechanism.⁹² These channels can be blocked by a class of short peptides known as conotoxins, but these have not yet been evaluated as potential neuroprotectives.⁹³ Intracellular calcium is also mobilised during

excitotoxic overstimulation and inhibitors of the release of intracellular calcium, such as dantrolene, are neuroprotective in tissue culture. Increases in intracellular calcium may activate lipases, proteases and endonucleases resulting in the condensation of nuclear chromatin and DNA fragmentation, leading to apoptosis or cell death. Other potentially cell damaging systems initiated include protein kinase C, phospholipases, and nitric oxide synthase. Activation of phospholipase A₂ generates arachidonic acid and its metabolites as well as platelet activating factor. This in turn potentiates the glutamate induced potentials, reducing astrocyte glutamate reuptake mechanisms and increasing neuronal calcium.⁹⁴ Thus self perpetuating mechanisms of increasing levels of intracellular calcium and glutamate lead to exacerbation and expansion of the area of neuronal injury and cell death. The homeostasis and pathological effects of excess glutamate and calcium are summarised in figure 1.09.4.

Nitric oxide and cerebral vasculature

Nitric oxide has a complex role in the regulation of the cerebral circulation. It may act as a neurotransmitter and also controls vascular tone. Vasodilatation to carbon dioxide and neurotransmitters such as acetylcholine, serotonin, substance P, and ADP are dependent on the generation of NO.⁹⁵⁻¹⁰² NO is produced by a variety of cells within the cerebral circulation. Neurones, glia and endothelium can all produce NO mediated by the enzyme nitric oxide synthase (NOS) which converts L- arginine to citrulline and NO. In conditions of L-arginine deficiency NOS may however lead to the production of toxic free radicals such as superoxide. NO acts on local target cells by activating guanylate cyclase (GC). NO may be present and active under basal conditions in a constitutive form, the generation of which may be further stimulated by increases in intracellular calcium. Much larger quantities of inducible NO can be produced and this may mediate more pathological effects.^{103,104}

In vivo administration of inhibitors of NO synthase, i.e. the L-arginine analogues L-NMMA, L-NNA and L-NAME, all reduce local cerebral blood flow by increasing local vascular tone. Impaired endothelium dependent relaxation is also observed in pathological states such as hypertension¹⁰⁵⁻¹⁰⁸ and diabetes.¹⁰⁹⁻¹¹¹ This appears to be due to the production of an endothelium derived contracting factor that counteracts the normal physiological dilator effects of NO. Activation of the NMDA receptor complex induces production and extracellular release of NO by neurones.¹¹² Administration of excitatory amino acids such as glutamate also induces cerebral arteriolar dilatation through a NO dependent pathway.¹¹³⁻¹¹⁶ Nitric oxide may have both beneficial and toxic effects in cerebral ischaemia, as demonstrated by the conflicting reports of neuronal culture and in-vivo experiments. On the benefit side NO increases local perfusion, by inducing vasodilatation and inhibiting aggregation and adherence of platelets and polymorphs.¹¹⁷ Higher levels may however be toxic and it is postulated that the beneficial or toxic effect is determined by the redox state of NO. Oxidised NO produces the nitrosonium ion which reacts with the thiol group on the NMDA receptor's modulatory site. This reaction acts as a negative feedback mechanism downregulating the NMDA receptor and preventing excess stimulation and excitotoxicity. The reduced form however may react with superoxide anions to produce a neurotoxic peroxynitrite ion (ONOO^-).¹¹⁸

In short, processes involving NO are important regulators of the cerebral circulation and in addition have a paradoxical role in the amelioration and exacerbation of excitotoxic mediated neurotoxicity dependent on redox state.

1.10 Thrombolysis

In patients with acute myocardial infarction thrombolysis is now known not just to improve mortality but also to preserve left ventricular (LV) function,^{119,120} with a window of opportunity of at least 12 hours. The idea of restoration of blood flow as soon as possible following the onset of symptoms forms the basis for the current management of myocardial infarction and it was hoped that the same 'open artery hypothesis' could be applied to patients with acute stroke. A number of trials of thrombolytic treatment have now been concluded and the results are discussed below.

Prospective analysis of spontaneous reperfusion rates in patients with stroke suggests early reperfusion of viable tissue is associated with smaller infarcts and a better prognosis.¹²¹ The data published thus far are confusing in part because inclusion criteria and concomitant anticoagulant therapy affected the rate of haemorrhagic transformation and consequently altered outcome. The overall picture suggests that early thrombolysis, within 3 hours of the onset of symptoms is associated with a better long term (after 3 months) outcome than no treatment at all.

The NINDS study was a multicentre randomised placebo controlled study of thrombolysis with recombinant tissue plasminogen activator (rt-PA) within 3 hours of onset of symptoms in patients with CT confirmed ischaemic stroke. Results reported that patients receiving thrombolysis were 30% more likely to have minimal or no disability at three months (figure 1.10.1). This improvement in outcome was partly offset by a 10 fold increase in the rate of symptomatic intracerebral haemorrhage (6.4% vs 0.6%). Despite this high incidence of secondary haemorrhage the mortality figures were similar in the two groups (17% in the thrombolysed group vs 21% in the placebo group).⁴

Other studies evaluating either a longer time window with rt-PA or streptokinase therapy at any stage have reported either negative results or at best a trend towards efficacy. What has been reported consistently is an increase in the number of symptomatic cerebral haemorrhages in the treated groups and a resultant increase in early mortality (figures 1.10.2 and 1.10.3).

The Multicentre Acute Stroke Trial-Europe (MAST-E) was discontinued prematurely because of safety concerns.¹²² These results precipitated early analysis and discontinuation of the Australian Streptokinase (ASK) study. In both cases, administration of streptokinase (within 4 hours in the case of ASK and 6 hours in MAST-E) was associated with an increased incidence of intracerebral haemorrhage. In the ASK study the odds ratio of an adverse outcome (death or poor functional outcome) was 2.65 in patients treated 3-4 hours after stroke.¹²³ MAST-E reported an increased rate of symptomatic haemorrhage in thrombolysed patients (17.5% vs 5%) and this corresponded with a significantly higher early mortality rate in actively treated patients (44% higher mortality at 6 months). In this study there was a particularly high rate of concomitant prescription of anticoagulants (65% in the active and 75% in the placebo arm) and this may have influenced outcome adversely.¹²⁴

The MAST-I study was designed to assess both aspirin and thrombolysis as potential acute stroke therapies. The study had a 2 x 2 factorial design with patients randomised to streptokinase with or without aspirin, aspirin alone, or placebo. This study was terminated after safety analysis of data from 600 patients out of a total of 1500 proposed. Again there were safety concerns as early death was more common in the thrombolysed patients with an additive effect in those patients also receiving aspirin (10 day fatality odds ratio 2.7). A small beneficial effect on late mortality and severe disability was noted but this may reflect the higher early death of more severe stroke patients. This positive effect was at best marginal and could not be used to advocate thrombolytic treatment. Aspirin alone was not associated

with poor outcome, early or late and there was a non significant trend toward efficacy in this group.^{125,126}

The European Cooperative Acute Stroke Study (ECASS) failed to demonstrate a benefit of rt-PA (according to the study's predesignated primary hypothesis) in patients within 6 hours of stroke but the study did raise a number of issues of relevance to the interpretation of other trial results. This study intended to recruit only patients with moderate-severe stroke but without signs of major early infarct on CT scanning. Despite strict entry criteria and only specialised centres being used for recruitment, 17.4% of patients were retrospectively excluded from the target population analysis. Forty out of sixty of these exclusions in the actively treated group were due to ineligible CT scan criteria i.e. 31 major infarcts, 2 haemorrhages and 7 unavailable. It is notable that this subgroup had the worst survival outcome.

There was no improvement in functional outcome in the intention to treat analysis but exclusion of ineligible patients in a target population analysis demonstrated a significant improvement in functional outcome in rt-PA receiving patients ($P = 0.035$) with no difference in mortality in either analysis.¹²⁷ Significantly there was a far higher proportion of patients with complicating intracerebral haemorrhage in both placebo and actively treated patients within the ECASS study compared with NINDS. This may have been due to better patient selection in NINDS but may simply have occurred by chance. The result was higher absolute numbers of complicating haemorrhages in the ECASS rt-PA group, and a statistically insignificant increase in patient deaths.

It could be argued that the longer time window in the ECASS study allowed entry of patients beyond a therapeutic window of opportunity, thereby diluting any beneficial effect and making haemorrhagic transformation more likely. Further analysis of the ECASS data however do not demonstrate poorer functional outcome in patients treated between 3 and 6 hours

compared with those treated within 3 hours, but this represents a post-hoc analysis, and as such is not wholly reliable.

NINDS used a lower dose of rt-PA than ECASS and this also may have contributed to the higher incidence of haemorrhages in the ECASS study. If we examine the outcome criteria assessed in the two trials we find a crucial difference. NINDS dichotomised outcome measures into categories of good outcome vs poor outcome. Good outcome was defined as a Barthel¹²⁸ of at least 95/100, a Rankin score of ≤ 1 , or a NIH Stroke Scale¹²⁹ of ≤ 1 (all equivalent to minimal deficit). Poor outcome was defined as anything else. The ECASS study however set out to prove the hypothesis that rt-PA would improve functional outcome by > 15 points on the Barthel or > 1 on the Rankin scale at 90 days. When the data acquired from the ECASS study are reanalysed using the NINDS hypothesis to assess outcome there is a significant benefit from rt-PA in terms of Rankin and NIH stroke scale but only a non-significant trend to improvement in Barthel index (figure 1.10.4). A further ECASS study is underway investigating the lower dose of rt-PA and with more cautious selection of patients. In a recent meta-analysis of thrombolytic therapy the authors suggest there is little evidence that rt-PA is safer than SK and suggest further trials of SK are merited. This conclusion is surprising as all the evidence suggests that SK increases early mortality and that the incidence of complicating cerebral haemorrhage is substantially increased.¹³⁰

We could postulate from these results that while very early thrombolysis may be of benefit any delay beyond 3 hours tips the balance towards an unfavourable outcome. PET scans in stroke patients demonstrate that reperfusion of areas of brain which do not extract oxygen or metabolise glucose does not lead to smaller infarcts compared with the beneficial effect of reperfusion in areas which have increased levels of extraction. Reperfusion of ischaemic brain where there is no increase in the extraction fraction of oxygen conversely reflects reperfusion of unsalvageable tissue, or 'luxury perfusion'.¹³¹ This paradoxically leads to

additional necrotic damage or 'reperfusion injury' due to the influx of free radicals and polymorphs. Single Positron Emission Computed Tomography (SPECT) studies suggest reperfusion of areas with low residual cerebral blood flow is associated with an increased risk of haemorrhagic transformation¹³² and this may explain the poorer outcome in patients thrombolysed beyond the 3 hour time window. What is therefore required is a form of diagnostic imaging which can predict whether or not tissue is potentially salvageable prior to the administration of thrombolysis. At present PET scanning is not routinely available and certainly could not be performed within the 3 hour time window postulated. We are left with the conclusion that as current evidence stands thrombolysis is not a practicable treatment for the majority of patients with acute stroke. The NINDS study does however demonstrate for the first time in a randomised, controlled study, that therapeutic intervention can benefit outcome in patients with stroke. In the United States rt-PA is now licensed as treatment for acute stroke within 3 hours of presentation but is not in general use in Europe.

1.11 Aspirin, antiplatelet agents and anticoagulation

Aspirin

Two recently completed controlled trials of immediate aspirin therapy recruiting 40,000 patients have demonstrated a small but significant benefit from early aspirin use in acute stroke. The International Stroke Trial (IST)¹³³ and Chinese Acute Stroke Trial (CAST)¹³⁴ together suggest a benefit of about 10 deaths per 1000 if aspirin is commenced immediately. This benefit was offset by a small increase in the incidence of complicating intracerebral haemorrhage (2 per 1000). There was also a modest improvement in functional outcome in the aspirin receiving patients, with 11(SD 6) fewer patients dead or dependent at discharge within the CAST study and 13 per 1000 less at 6 months in the IST. The conclusion of both trial groups was that aspirin was safe and efficacious within the first weeks after stroke. The results reassure stroke physicians, most of whom have been prescribing aspirin acutely after

the exclusion of cerebral haemorrhage. It is therefore unlikely that the results of these studies will have a significant impact in terms of altering current patient management.

The International Stroke Trial also examined the effect of acutely prescribed heparin (12500u bd or 5000u bd). There was no reduction in deaths at 14 days or death and dependency at 6 months. Again a small benefit in recurrent early stroke was offset by an increase in the risk of haemorrhagic stroke. Crucially, entry to the trial was left to the discretion of the physician with no detailed inclusion and exclusion criteria. This introduces potential bias which could mask a significant risk or benefit in the patients excluded from the study.

A number of trials of fractionated heparin are underway. Two doses of nadroparin were assessed in a recently reported study. The higher was equivalent to full anticoagulation for DVT and the lower equivalent to prophylactic use. The results suggest benefit of high dose low molecular weight heparin with a 30% reduction in the relative risk of a poor outcome compared with the placebo group (death or dependency at 6 months). This corresponded with a risk ratio of 0.69 (95%CI 0.54-0.90). There was an trend toward reduction in poor outcome within the lower dose group. No increase in the overall incidence of adverse events was reported in the active treatment arms and there was no evidence of a substantial risk of haemorrhagic transformation.¹³⁵

This study raises some interesting issues for future research. Are fractionated heparins effective and safe in stroke patients and is there a significant dose effect? This could partially explain the failure of the IST to produce a beneficial effect.

Anticoagulants and secondary prevention for stroke

The reported results of five recent trials suggest the relative safety and benefit of anticoagulation in patients with non-rheumatic atrial fibrillation. Warfarin reduced the risk of stroke from 4.5% to 1.4% per year (risk reduction 69%)¹³⁶⁻¹⁴⁰ This was associated with a small increase in the incidence of haemorrhage. The second Stroke Prevention in Atrial Fibrillation Trial (SPAF II) compared aspirin (325 mg) and warfarin (INR 2- 4.5) in patients with non-rheumatic atrial fibrillation. Aspirin appeared as effective as warfarin in preventing large disabling strokes. Warfarin did reduce the total number of strokes but was associated with a higher incidence of intracerebral haemorrhage.¹⁴¹

In younger patients with few risk factors for thromboembolism, the absolute risk of stroke is low and there is little additional benefit in full anticoagulation compared with aspirin. In contrast, older patients and those with previous TIA or stroke, hypertension, heart failure, previous thromboembolism or an abnormal echocardiogram have a higher annual incidence of stroke and would probably benefit from warfarin treatment. A trial of warfarin (target INR 3.0 - 4.5) versus aspirin for secondary prevention of patients without cardioembolic disease was abandoned when the risk of haemorrhage within the warfarin-treated group was found to be excessive (KR Lees, personal communication). A further trial (ESPRIT) is now underway in more cautiously selected patients, testing lower dose warfarin (target INR 2.0 - 3.0) versus low dose aspirin, versus aspirin plus dipyridamole.

Patients with atrial fibrillation without cerebrovascular disease therefore require individual risk factor assessment for both stroke and complicating haemorrhage and before allocation to either warfarin or aspirin therapy as appropriate. Once patients have suffered even a transient ischaemic neurological event they immediately become high risk and should benefit from full anticoagulation.

Other anti-platelet agents

Ticlopidine and clopidogrel inhibit the binding of the platelet agonist ADP. This in turn inhibits the activation of the GP IIb-IIIa complex, the major receptor for platelet activation. A recent study compared ticlopidine with placebo in patients with known peripheral vascular disease but crucially did not compare ticlopidine with current accepted therapy (aspirin). A dramatic reduction in the incidence of myocardial infarction resulted in a 29% reduction in mortality in the ticlopidine receiving patients ($p=0.015$), but 2% of patients developed significant haematological abnormalities and there was also an excess of gastro-intestinal upset in the actively treated patients.¹⁴² While ticlopidine is likely to be an effective anti-platelet agent the side effect profile and additional expense has precluded its introduction to the UK though it is commonly prescribed in the U.S.

Clopidogrel, while acting by the same mechanism, does not appear to cause haematological abnormalities. A recent direct comparison between clopidogrel and aspirin in patients with vascular disease suggested a significant annual relative risk reduction of 8.7% (95% CI 0.3-16.5). At present the additional expense of clopidogrel will prevent its use in place of aspirin as a first line anti-platelet. It may find use, however, as an add-on therapy in patients at high risk of vascular events.¹⁴³

The potential benefits of combination therapy were also examined within the second European stroke prevention study (ESPS-2).¹⁴⁴ This study suggested the combination of aspirin + dipyridamole was more effective in reducing the secondary incidence of stroke than either drug alone (additional risk reduction of 23.1% from combination therapy compared with aspirin alone). Dipyridamole and aspirin have different modes of action; dipyridamole inhibits aggregation by raising platelet levels of c-AMP and c-GMP whereas aspirin acts by irreversibly inhibiting platelet cyclo-oxygenase which in turn reduces thromboxane production. Since the ESPS-2 results support the hypothesis that antiplatelet agents with

different modes of action have additive effects it is likely that combination therapy will be increasingly researched and prescribed, as evidenced by the protocol of the ESPRIT study.

1.12 Neuroprotection

Preclinical Evidence

The most conclusive evidence that glutamate mediated activation of receptors is responsible for expansion of the infarct volume comes from animal models of acute stroke. In these studies, drugs antagonising the NMDA receptor complex consistently reduce the size of infarction induced by a standardised vascular or hypoxic brain insult.⁷⁸ The receptor complex itself is made up of several integral parts. Glutamate binds at a specific recognition site on the NMDA receptor. Competitive blockers of this site include CGS 19755 (selfotel) or cis-4-phosphomethyl-2-piperidine carboxylic acid and D-CPPene. Other potential sites for antagonism of the receptor include the ion channel that is blocked physiologically by magnesium or pharmacologically by aptiganel (chapter 7), remacemide (chapter 5) and dizocilpine (MK801); and the glycine site, since glycine binding is required for the activation of the receptor, blocked by GV150526 (chapter 6) and ACEA1021. Clinical studies are currently underway to assess tolerance and efficacy of these drugs in patients with stroke. In addition, a polyamine site distinct from the ion channel but part of the NMDA receptor complex (possibly the NR2B subunit) has been identified and antagonists are currently in clinical development.⁸¹ GABA-A receptors have important inhibitory functions within the CNS and a number of agonists have been assessed in models of focal ischaemia. GABA administration blocks the excitotoxic effects of glutamate including depolarisation and calcium influx. GABA-mimetic drugs such as chlormethiazole or muscimol are neuroprotective and combination therapy with the NMDA antagonist MK 801 has been shown to be more effective than treatment with chlormethiazole alone. Unfortunately the high incidence of respiratory depression with chlormethiazole was also potentiated by MK

801.^{145, 146} Potential sites of pharmacological interaction with the NMDA receptor are shown in figure 1.12.1.

Neuronal Vacuolation

Despite being shown to be effective in animal models of cerebral ischaemia there remain some safety concerns regarding this group of drugs: PCP and other NMDA antagonists have been shown to induce a neurotoxic reaction within the cingulate and retrosplenial cortex of the adult rat.¹⁴⁷ Mitochondria and endoplasmic reticulum are acutely transformed into large intraplasmic vacuoles.¹⁴⁸ It has been claimed on the one hand that these changes are completely reversible¹⁴⁹ and on the other that prolonged administration may result in neuronal death.¹⁵⁰ Reassuringly no NMDA antagonist currently in development for stroke has been associated with permanent neuropsychiatric effects or pathological changes in humans. Partly because of these pathological findings and the recognised physiological role of glutamate in neurological function there is concern that administration of NMDA antagonists, particularly if prolonged, may lead to impairment of learning and cognition.

The NMDA antagonist remacemide (chapter 5) has however been used in the treatment of epilepsy chronically with no significant permanent neurological or psychological effects. It is therefore unlikely that this effect will prejudice the development of therapeutic agents.

Neuroprotection in stroke patients

Stimulation of the NMDA receptor leads to the initiation of the excitotoxic positive feedback system and a large number of inhibitors of the receptor complex are in various stages of development. Trials with selfotel in traumatic brain injury and stroke were abandoned in late 1995 due to lack of a favourable risk-benefit profile.¹⁵¹ Theoretically the competitive block

induced by these drugs can be overcome by increasing concentrations of glutamate within the ischaemic brain, which could potentially cause a reduction in efficacy. The prototypic compound MK 801 and other subsequently developed blockers apiganel or phencyclidine (PCP) do not suffer from this potential drawback but at high doses may give rise to psychotomimetic or hallucinatory side effects.⁸¹

Another potential hazard of this group of NMDA antagonists is haemodynamic changes. These issues are discussed in detail above and also in chapter 7. Any drug being evaluated as a stroke therapy must have its haemodynamic side effect profile assessed.

Glycine is a co-agonist at the NMDA receptor and antagonists of the glycine binding site are currently in clinical trials. GV150526 appears to be well tolerated with a favourable side effect and safety profile compared with glutamate site inhibitors (chapter 6). Glutamate release itself may also be inhibited and the Wellcome release inhibitor 1003C87 has been shown to be neuroprotective in a MCA occlusion rat model without being associated with the vacuolation changes noted with NMDA antagonists.⁸¹ The Glaxo Wellcome compound 619C89 blocks veratrine-induced release of glutamate. It has been evaluated in animals and has now progressed to phase II clinical trials.¹⁵²⁻¹⁵⁴ Other drugs may inhibit glutamate release indirectly via their action on sodium channels i.e; phenytoin, riluzole, lamotrigine, lifarizine.⁸¹ Adenosine inhibitors may also have neuroprotective activity through this same mechanism but there are no details as yet on tolerability at experimentally effective doses.¹⁵⁵

The polyamine site (made up of the molecularly isolated NR2B subunit) is a further allosteric site within the NMDA receptor complex. Ifenprodil and eliprodil (SL 82.0715) are antagonists at this binding site. Development of eliprodil was halted due to a lack of clinical efficacy, though at high doses drug was noted to cause QT prolongation due to its mode of action (blocking voltage sensitive calcium channels).¹⁵⁶

The opening of the NMDA receptor ion channel that facilitates the influx of calcium is voltage dependent and is blocked in physiological conditions by magnesium. Ischaemia induced excitotoxic depolarisation removes the magnesium dependent block. Magnesium in the form of the chloride and sulphate salts has been shown to be neuroprotective in permanent MCA occlusion models of cerebral ischaemia.¹⁵⁷ It is cheap to produce, and appears well tolerated in initial pilot studies in stroke patients.¹⁵⁸⁻¹⁶⁰ It has already been safely given to over 50,000 patients with acute myocardial infarction¹⁶¹ and is currently being evaluated in a multicentre randomised, placebo controlled trial (IMAGES).¹⁶² Results in stroke patients suggest it is well tolerated with no adverse effects on blood pressure.¹⁵⁹

AMPA receptors are also activated by excess excitatory amino acids and antagonists are currently being developed. AMPA receptor blockers are more effective than NMDA receptor agonists in some animal models of global cerebral ischaemia^{163,164} and combination blockade of NMDA and AMPA together appears to be synergistic, although associated with respiratory depression.¹⁶⁵

Free radicals are thought to have a role in mediating ischaemic neurone damage and in particular reperfusion injury. A free radical is a reactive species with an unpaired electron. Once generated, free radicals rapidly react with other molecules forming more free radicals and setting up a damaging chain reaction. Free radicals may react with cellular constituents i.e. nucleic acids, lipids, and particularly the polyunsaturated fatty acids within neuronal membranes, damaging both the membranes themselves and associated receptors.¹⁶⁵ The combination of ischaemia, a rich supply of metal complexes (i.e iron from haemoglobin), and a paucity of free radical neutralising enzymes (superoxide dismutase, catalase and glutathione peroxidase) within the brain predisposes to neurone damage mediated by free radicals. Vitamins C (ascorbate) and E (alpha-tocopherol) are naturally occurring free radical scavengers, but are also unfortunately relatively deficient within the central nervous system.¹⁶⁶

Various means of reducing free radical damage during and after cerebral ischaemia are being preclinically and clinically evaluated. The synthetic 21-amino-steroid tirilazad has free radical scavenging activity, analogous to Vitamin E. It also has anti-oxidant effects, inhibiting the generation of hydrogen peroxide and blocking the release of arachidonic acid from injured cell membranes. It is effective in animal models of stroke, reducing the volume of infarction in the rat MCA occlusion model, but so far phase III clinical trials have been disappointing.¹⁶⁷ Enzymes like superoxide dismutase (SOD) can convert unstable free radicals to more stable, less harmful molecules. Unfortunately these do not directly cross the blood brain barrier, but conjugation to lipid soluble agents i.e. polyethylene glycol may allow blood brain barrier penetration. These mechanisms are currently undergoing preclinical evaluation.¹⁶⁸

Neutrophils also have a role in the development and maturation of cerebral infarction and mediate some aspects of reperfusion injury.¹⁶⁹ Neutrophil adhesion is mediated by specific adhesion molecules which are essential in initiating the release of cytotoxins and in controlling cellular activation. Monoclonal antibodies to these adhesion molecules (i.e. anti-CD11 and anti-CD18) also reduce infarction volume but are unsuitable for clinical use, due to their immunogenicity.¹⁷⁰⁻¹⁷² A recent study has shown the effectiveness of a recombinant neutrophil inhibitory factor derived from hookworms in reducing infarct volume following MCA occlusion in rats. This correlated with a reduction in the number of neutrophils found within the infarcted tissue.¹⁷³ Unfortunately, results of a phase-3 trial with the anti-ICAM drug enlimomab, are reportedly negative; indeed, mortality was higher in the actively treated group, perhaps due to increased inflammation (D Sherman, oral communication). It is possible that immunological therapies will also have a broader window of opportunity as they modulate more delayed effects of infarction. Studies have evaluated treatment following 2 hours of middle cerebral artery occlusion in rats. Further studies should evaluate the

possibility of significant neuroprotection beyond the time window already established for NMDA antagonists in animal models.

1.13 Therapeutic window of opportunity and duration of therapy

PET studies suggest ischaemic but still potentially viable infarct zone tissue may be present up to 48 hours after stroke, but it is not yet proven that ischaemic brain tissue is potentially salvageable beyond the 3 hour time window demonstrated in the NINDS trials of thrombolysis. Forty eight hours may be an overoptimistic time window and 6-12 hours is probably nearer the true time limit for useful neuroprotection in patients. The outer limit of the therapeutic time window will be established only by large scale trials of a proven therapy which does not carry the risks that attach to thrombolysis. It is likely that neuroprotective treatment will not be the two edged sword that thrombolysis has proven to be. While it is true that excitotoxic damage appears to be initiated rapidly after stroke onset delayed administration of these drugs should not be associated with the reperfusion complications of cerebral oedema and cerebral haemorrhage which are responsible for poor outcome in patients thrombolysed beyond 3 hours.

As discussed above, a number of neuroprotective compounds are in advanced stages of clinical development following encouraging results from preclinical studies. There is however no consistent approach to dosing schedules for these novel treatments and as a result ongoing phase three clinical studies of efficacy are using differing and possibly inappropriate durations of drug therapy. In most phase three efficacy studies, drug is administered as soon as possible after stroke within a predetermined but arbitrary time window. Drug is then often continued for a variable time depending on the pharmacological properties of the individual compound. For example, studies with tirilazad or selfotel (CGS19755) have restricted recruitment to cases that can be treated within 6 hours. In the case of selfotel, the drug was given as a single intravenous bolus¹⁷⁴, whereas tirilazad administration was repeated for 72

hours.¹⁷⁵ In contrast, piracetam efficacy was assessed when commenced within 12 hours and continued for up to 12 weeks after the onset of symptoms.¹⁷⁶

It is clear that in both animal models of stroke and humans the effects of cerebral ischemia are manifest on the cerebral metabolism rapidly, within a timescale measured in minutes or hours.^{67,79,177} Any form of potential neuroprotective treatment should therefore be given by the most rapidly effective route. In practice, this means intravenously. In an ideal scenario, neuroprotective plasma and CNS levels of drug would be attained immediately. This is widely recognised, and is supported by the results of the recent thrombolysis trials [NINDS, ECASS, MAST, MAST-II]^{4,122,126,127} and by meta-analysis of nimodipine trial results.¹⁷⁸ Questions which remain, and which require attention before that magic bullet is found, concern the issues of how late after stroke can treatment of any kind be usefully administered and how long and by what route subsequent therapy should be given.

The available human PET data suggests a rationale for initiating and continuing neuroprotective treatment up to at least 48 hours after stroke onset, as within this time window metabolically compromised ischaemic brain has been shown to return to normal. Also regional blood flow abnormalities tend to resolve in most cases within 3-4 days due to reperfusion via collateral vessels which develop in the intervening time. During this time cerebral autoregulation is deranged and as a result cerebral perfusion is determined by systemic BP. There is new evidence that suggests EAA's remain grossly elevated for at least 6 days after stroke.¹⁷⁹ These data were gathered using a microdialysis probe inserted into an area of infarcted tissue during a neurosurgical procedure to relieve raised intracranial pressure following a large cerebral infarct. The conditions of the study were therefore atypical and the results require confirmation. If we consider this evidence we can conclude that for 3-4 days the cerebral circulation is in a state of turmoil whereby autoregulation is deranged and collateralisation and reperfusion may occur. It would seem logical to protect patients with some form of neuroprotective therapy during this period i.e. for at least 3-4 days.

If further evidence confirms elevated EAA's at 6 days or beyond a case could be put for more prolonged treatment.

The precise timing of these events and the status of the patient's cardiovascular, respiratory and cerebral circulation will determine the extent of the eventual stroke deficit. While early collateralisation may lead to improved outcome and late collateralisation to reperfusion injury physicians cannot yet predict which outcome is likely in individual patients. It is predominantly this inability which makes thrombolysis such a hazardous treatment.

The route and means of administration of treatment will depend on the individual pharmacokinetic properties of the neuroprotective compound, on the adverse effect profile of the drug, and on the nature of the insult giving rise to the stroke. For example, it would be desirable to maintain neuroprotection throughout recurrent episodes of cerebral ischemia as seen in association with recurrent cardiac embolism. While this risk may vary from patient to patient, phase three studies should address the safety and efficacy of durations of treatment that are likely to be utilised subsequently in routine clinical practice.

Pharmacological properties

Ideally, any compound for the treatment of stroke should adequately cross the blood brain barrier, and obtain sufficiently therapeutic levels rapidly within the brain and CSF. Highly lipid soluble drugs will penetrate the cerebral tissues more rapidly than hydrophilic agents and will also be cleared more slowly from neural tissue. Although there are scant data available in human subjects, investigations of the pharmacokinetics of the lipid soluble neuroprotective agent selfotel in human volunteers, suggest that the brain half life of drug is significantly longer than the plasma half life. Selfotel was present in the CSF after 16 hours in contrast to its plasma half life of 2-3 hours.¹⁸⁰ The central nervous system effects were also prolonged: up to 60 hours in patients with stroke. The active drug pool need not

necessarily be within the CSF, however, and tissue levels are likely to be more important since CSF may equilibrate only slowly with brain tissue.

Drugs with slow clearance from the brain tissue are more likely to accumulate and lead to toxic side effects if given by constant i.v infusion but conversely may give prolonged protection if given by single bolus injection.¹⁸⁰ Overall, lipid soluble agents are more likely to exhibit in-vivo activity at relatively lower plasma levels than water soluble agents and drug doses required for neuroprotection may be overestimated by plasma level calculations and pharmacokinetic modeling. Maintenance doses may not even be required to sustain neuroprotective drug levels within the brain.

At present little is known about the effect of stroke on the integrity of the blood brain barrier, which is crucial in determining the penetration of less lipophilic compounds. In acute cerebral ischemia it is possible that hydrophilic compounds may cross the blood brain barrier and thus enter infarcting or ischemic tissue. It is also important to consider the limitations of calculating maintenance drug doses from data based on plasma levels of drug and or metabolite, which of course give no indication of CSF and brain levels of drug during treatment. Studies in which patients are subjected to frequent removal of CSF via lumbar puncture following infusion of neuroprotective agents are unacceptable to most physicians and patients. There are limited data regarding the CSF or brain penetration of these compounds in man and thus the quantity of drug actually reaching the brain is unknown. Positron emission tomography (PET) studies using isotope-labelled drug may give some indication of drug distribution in man, but there are considerable practical difficulties involved. Patients must be recruited, examined, scanned with CT or MRI, treated and then undergo PET scanning, all within several hours of stroke onset.

Binding properties

At a cellular level the mechanism of action of any particular agent will also determine whether constant exposure to drug is necessary or indeed desirable. In the case of non-competitive high affinity NMDA antagonists such as aptiganel, binding occurs rapidly if the ion channel is open and the drug dissociates slowly.⁸¹ Thus after dosing, increasing numbers of ion channels become blocked over time until steady state is reached. Conversely, lower affinity blockers such as remacemide (chapter 5) will dissociate from the receptor more readily and thus may require a higher loading dose followed by a maintenance infusion to achieve effective ion channel blockade.

The clearance and volume of distribution of any given compound will influence the doses required but not necessarily the duration of treatment. Where the half life of drug is relatively long (e.g selfotel) it may be more acceptable to patients and medical staff to give treatment in the form of single or intermittent iv bolus, rather than a constant iv infusion. The exception is in the case of drugs which have a narrow therapeutic index, i.e. for which the minimal effective plasma concentration is close to the maximum tolerated plasma concentration. In this case, despite a long half-life, it may be impossible for patients to tolerate the peak concentrations achieved after each bolus unless doses are so low that the trough concentrations may be ineffective. An example is aptiganel which in phase II studies was given as initial bolus followed by constant i.v infusion, as initial peak concentrations following higher single bolus injection were associated with intolerable side effects. Tolerability may therefore be improved by minimising fluctuations in drug concentration. Initial dosing schedules for the glutamate release inhibitor 619C89 utilised intermittent dosing whereas more recent studies have relied on constant rate infusion.¹⁵⁴

Most compounds investigated as possible treatments for stroke in animal models are maximally effective given as initial bolus followed by constant i.v infusion. The selective competitive NMDA antagonist EAA090 paradoxically is maximally effective after single i.v bolus only (Dr P Danjou, personal communication). The significance of these differences in humans is unknown and may be marginal or even irrelevant but may influence the dosing schedules that are chosen for the evaluation of therapies in future clinical trials.

The therapeutic range, and thus the desirable duration of therapy, is also influenced by the steepness of the dose-response relationship. The effects of aptiganel in healthy volunteers include subjective oral paraesthesia and at higher doses, objective evidence of nystagmus. The dose - response curves differ for these two effects (figure 1.13.1). Which of these effects is more closely related to neuroprotection is unknown, though the paraesthesiae appear to occur at plasma levels lower than have been associated with experimental neuroprotection. If neuroprotection were associated with the paresthesiae, then frequent or repeated dosing may be unnecessary, since a degree of neuroprotection would persist for many hours after a single bolus dose of aptiganel. Conversely, if neuroprotection is associated with nystagmus, then it is likely that plasma concentrations would fall below those required for efficacy within minutes to a few hours of ceasing aptiganel infusion.

Orally active drugs with good bioavailability characteristics may be suitable for longer term post-ischaemia treatment in patients with a high risk of imminent cerebral infarction. It is however common clinical practice to withhold food, drink and oral medications until speech and swallowing have been adequately assessed by a trained speech and language therapist, as swallowing is frequently compromised in patients with recent stroke.⁵⁵ It is therefore likely that early treatment of acute cerebral ischaemia will be limited to iv therapy.

It is likely that the lack of efficacy of thrombolysis beyond 3 hours is at least in part due to reperfusion injury. As both free radical scavengers and leucocyte adhesion inhibitors may reduce reperfusion injury, they should be considered as possible adjuvant therapy in combination with thrombolytic treatment. Reperfusion promoted by thrombolytic drugs probably occurs within at most a few hours of drug administration and spontaneous reperfusion up to a few days after occlusive stroke. If combination therapy with a neuroprotective agent and a thrombolytic drug is contemplated, then it should be sufficient to administer both drugs together, and to maintain treatment with the neuroprotective agent for two to three days.

Side effect profiles and safety

While it may be appropriate in some cases for neuroprotective therapy to be continued for hours, days or even weeks following acute ischaemic insult a paramount consideration is the tolerability and patient acceptance of any potential therapy. A number of neuroprotective agents have already been shown to have dose and duration-limiting side effect profiles. In particular, the more potent NMDA antagonists are associated with severe psychotomimetic effects. Aptiganel may cause lightheadedness, dizziness, paraesthesiae, sedation, and even paranoia;^{56,181} selfotel has been associated with agitation, confusion and hallucinations.

These symptoms have sometimes been reported as intolerable by patients receiving bolus or short infusion doses in clinical trials. Any clinically effective treatment even if poorly tolerated in the short term could potentially still be justified bearing in mind the poor prognosis of patients with moderate or severe stroke. Some of the NMDA antagonists may however be unsuited to maintenance infusion or repeated dosing because of the likelihood of prolonged severe side effects. Other NMDA antagonists in development, such as remacemide (chapter

5), appear better tolerated and have been given successfully with more acceptable levels of patient tolerability.¹⁸²

The glycine site antagonist GV150526 has a 'clean' side effect profile compared with antagonists acting at the ion channel pore of the NMDA receptor i.e.aptiganel. It may therefore be a suitable drug for longer term administration, although concerns regarding its effect on liver function remain (chapter 6).

Another potential neuroprotective agent with good tolerability is magnesium. Although the Intravenous Magnesium Efficacy in Stroke study (IMAGES)¹⁶² will be using only a 24-hour infusion, there is no reason why more prolonged treatment could not be administered.

Prolonged therapy for certain patients ?

The risk of further cerebral ischaemia following stroke or TIA is highest immediately after the initial event. Results from the Oxfordshire Community Stroke Project suggest the absolute risk of a further cerebral ischaemic episode is 4.4% during the first month and 8.8% in the first two months. The odds ratio of a stroke in these patients compared with age matched controls without recent symptoms of cerebral ischaemia is 80.0 within the first month and 27.0 within the second. Thereafter the odds ratio diminishes to 4.7 between 1 and 2 years.¹⁸³

While the risk of further stroke is relatively high in these patients, extrapolation of these results suggests that between 50 and 100 patients would require treatment for one week or alternatively 25 patients for one month to provide neuroprotection during a single recurrent event.

Patients with high grade stenosis of the internal carotid artery awaiting endarterectomy have a significantly higher risk of further recurrent stroke. In the control arm of the North American Symptomatic Carotid Endarterectomy (NASCET) trial the risk of stroke over a two year

period in patients with high grade ulcerative lesions was 30%.¹⁸⁴ The risk of recurrent brain embolism in the 14 days following cardioembolic stroke has been reported as 13.7% with the highest risk found in the 2 days immediately following stroke.¹⁸⁵ There are therefore subgroups of patients that can be identified as being at higher than average risk of further ischaemic events, who potentially could benefit from longer term neuroprotection. For prolonged treatment to be practicable it would ideally be available in an orally active form and have an acceptable side effect profile. Drugs could be given over a period of several days through the intra-venous route since anticoagulants are frequently administered in this way following a thrombo-embolic event. Potential interactions with drugs commonly used in the management of stroke patients, i.e. warfarin and aspirin, would however have to be assessed.

Practical issues of administration

For neuroprotective treatment to be effective it is likely that patients will require to have therapy initiated within at the most 12 hours after the onset of symptoms. Thus the healthcare infrastructure must facilitate the rapid referral, transfer to hospital, emergency assessment and treatment of such patients. Until now, with no proven therapy available for acute stroke, referral practices and assessment times are extremely variable both internationally and locally. The recent results with rt-PA are unlikely to change this situation, but should neuroprotection be demonstrated to be effective it is likely that this would improve, possibly with the development of a fast track referral system analogous to that in operation for patients with suspected myocardial infarction. It is conceivable that general medical practitioners or even paramedical staff could give an initial bolus dose of treatment prior to hospital transfer. Preparations of such drugs would need to be easy to administer and safe in patients with intracerebral haemorrhage, as clinical signs are unreliable in the diagnosis of this condition.¹⁰ The same caveat would apply to any form of therapy being

considered for use in smaller hospitals and isolated communities where there is no access to CT scanning facilities.

Potential problems

When considering how long intra-venous treatment may be continued, the potential effect on patient rehabilitation should be taken into consideration. Patients receiving intra-venous infusions are often immobilised as a consequence and this in itself may reduce the effectiveness of early attempts at rehabilitation. Furthermore, any treatment that immobilises patients following stroke is likely to lead to an increase in risk of thrombo-embolic complications, such as DVT. Prolonged infusions are associated with a risk of local phlebitis and indeed several agents under development are locally irritant.¹⁷⁵ It would therefore be ideal if treatment could be administered initially in an iv preparation and later converted to an oral formulation as soon as the patient was mobilised.

Drugs with potential respiratory depressant or sedative effects e.g. the AMPA antagonists¹⁸⁶ may cause practical problems in the management of stroke patients, in addition to increasing the risk of complicating aspiration pneumonia. It may be impossible to distinguish between the sedation caused by a complication of the initial stroke (eg secondary haemorrhage leading to raised intra-cranial pressure) and the sedation induced by the neuroprotective drug. Care must be taken that whenever possible appropriate concentrations of drug are prepared for the doses being considered, to avoid fluid overload: short term administration of high fluid loads (e.g 500mls in one hour) may be safe or even desirable in a dehydrated patient, but may precipitate incipient heart failure if prolonged in an individual with poor cardiac status.

In summary it is hoped that ongoing clinical trials will demonstrate efficacy of neuroprotective therapy in patients with acute stroke. When trials are conceived it is important to consider the way in which these drugs are likely to be utilised by physicians in the future and to design

studies accordingly. Side effect profiles of these agents require accurate definition and are crucial to the way in which drugs may be prescribed in the future: for example agents with potent adverse side effects i.e. psychotomimetic effects, may be suitable only for a single bolus or short term infusion. Well tolerated preparations could be given as repeat boluses or constant i.v infusion depending on the pharmacokinetic properties of the compound in question. The optimal duration of treatment is probably at least 72 hours, ensuring neuroprotection while cerebral hemodynamics are compromised. New evidence suggests EAA's may be grossly elevated for at least 6 days following large ischaemic stroke suggesting a possible rationale for more prolonged acute therapy.

Neuroprotective drugs may be combined in the future such that potent drugs with potentially upsetting side effects are given as an initial bolus and a better tolerated preparation used for prolonged therapy. Evaluation of the combination of rt-PA and neuroprotective therapy is inevitable now that rt-PA has become licensed within the U.S. Reperfusion injury may be reduced by administration of free radical scavengers or immunological modulators that reduce the influx and adherence of leukocytes in the infarct zone. Additional neuroprotection for high risk patients may continue if a suitable well tolerated orally active therapy was available. Ideally acute therapy would be available for administration by non specialist medical and paramedical staff as this would facilitate the earliest possible initiation of neuroprotection. Interactions of these agents with drugs frequently given following acute stroke will require assessment. Finally the relationship between acute changes in BP and stroke outcome requires further attention as some of these agents are likely to have haemodynamically significant effects.

1.14 Scope of thesis chapters

The work submitted for examination concerns several aspects of stroke patient management.

In chapter 2, I have examined the effect of the ACE inhibitor perindopril on blood pressure and total cerebral blood flow in hypertensive patients with recent stroke. It is still unclear at what stage it is safe to initiate antihypertensives but in most cases this should be delayed at least 72 hours. Most patients admitted to the Western Infirmary Stroke Unit are discharged either to the care of their General Practitioners or to a further in-patient facility (often rehabilitation) within 5-7 days of admission. It is therefore important to devise a risk factor intervention plan prior to discharge. Deferring a decision on blood pressure treatment until after discharge can result in unacceptable delay or even failure in the initiation of antihypertensive treatment. I hypothesised that ACE inhibitor treatment within 3-7 days of stroke onset was safe, effective and not associated with either exacerbation of neurological deficit, or reduction in cerebral blood flow.

In chapter 3, I have examined the relationship between cholesterol and outcome after stroke. This work initially began as an analysis of the risk factor profile of all admissions and outpatients under the care of our stroke service. This initial analysis suggested the incidence of raised cholesterol was identical to that of the local population. There appeared to be an excess of patients with very low cholesterol and intracerebral haemorrhage but this did not reach levels of statistical significance.¹⁸⁷ I went on to assess the influence of some of these risk factors on patient prognosis, with surprising results.

Less controversial is the relationship between poor stroke outcome and hyperglycaemia, that is further examined within chapter 4. Unlike the described relationship between better stroke outcome and raised cholesterol there are established biological mechanisms which explain

these observations. Again I found the relationship to be statistically significant and independent of other known prognostic variables.

Chapters 5, 6, and 7 were all phase II, placebo controlled trials of novel neuroprotective compounds being evaluated as treatment for acute stroke. The studies were not powered to demonstrate efficacy but rather to evaluate tolerability, safety and clinical pharmacology prior to a phase III study.

GV150526 is a glycine antagonist at the NMDA receptor complex. Prior to this study the compound had been administered to volunteers but not to patients. This early work had suggested good tolerability. The results confirmed this but suggested a drug effect on liver function. Remacemide has been extensively investigated as a potential anticonvulsant but also has significant neuroprotective effect in animal models of stroke. Unlike GV150526 it is known to have significant gastro-intestinal and CNS side-effects at high doses. A maximal tolerated dose was identified for further evaluation. Side effects were attributed to the build up of remacemide metabolites. While all the aforementioned work was solely conducted within the Stroke Unit of the Western Infirmary, the investigation of aptiganel in patients with acute stroke described in chapter 7 was carried out as a multi-centre study. This was an early phase II study. The work was carried out in two parts, the first to establish a safe and well tolerated loading dose, the second to examine tolerability of the selected loading dose followed by a constant infusion chosen from the pharmacokinetics established in part 1. Significant CNS dose limiting side effects were reported and furthermore haemodynamic effects requiring therapeutic intervention were observed.

Figure 1.01.1: Angiography of surgically correctable high grade internal carotid artery stenosis.

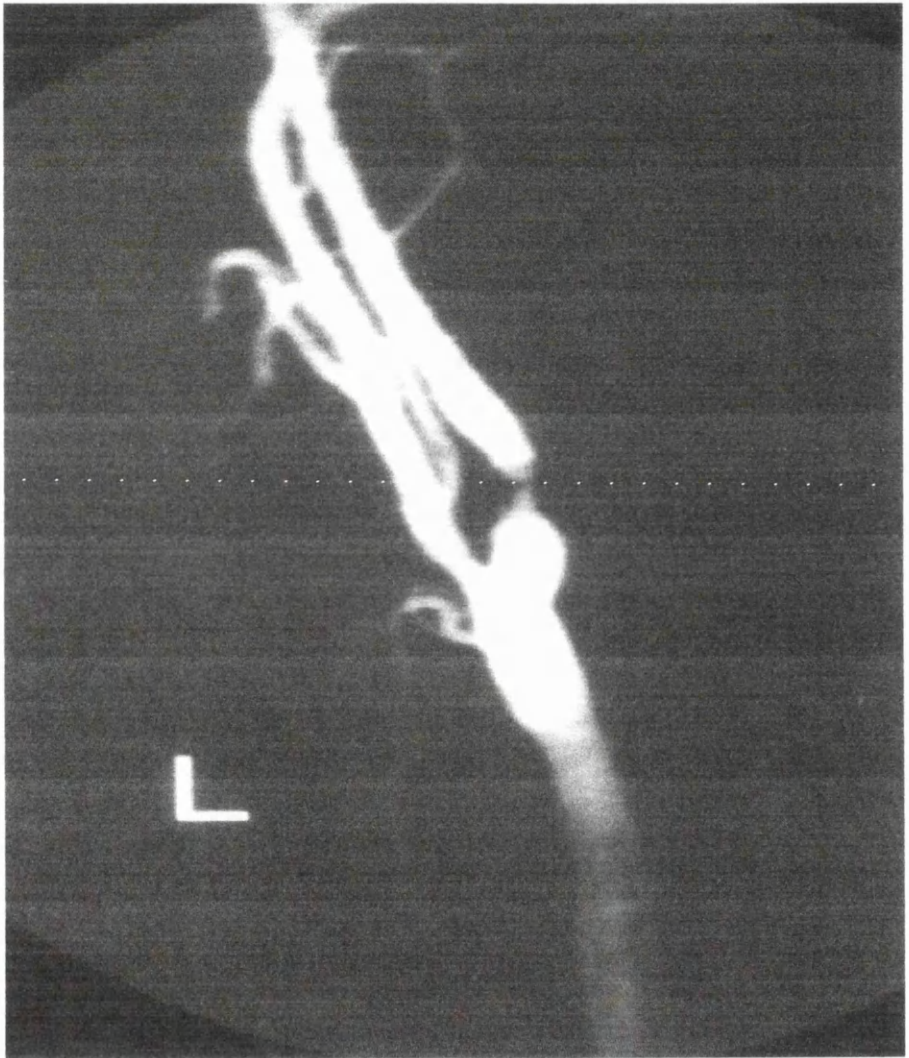


Figure 1.01.2: Doppler ultrasound in normal subject. The maximal velocity is below 1 m/second in the normal subject and there is no evidence of turbulent flow.

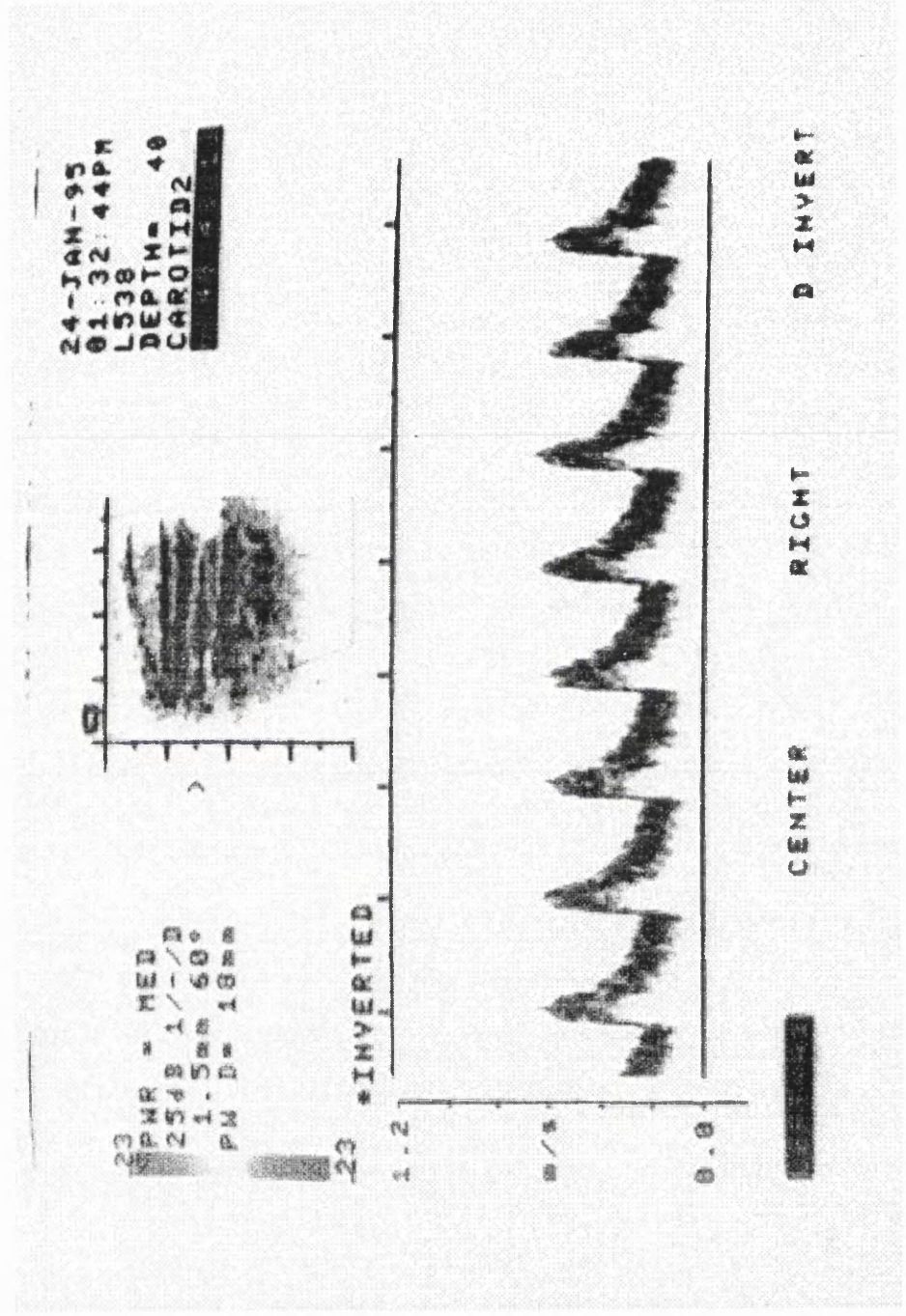


Figure 1.01.3: In contrast to previous figure, the maximal velocity is greatly increased in the presence of a significant carotid artery stenosis, and turbulence is present causing spectral broadening.

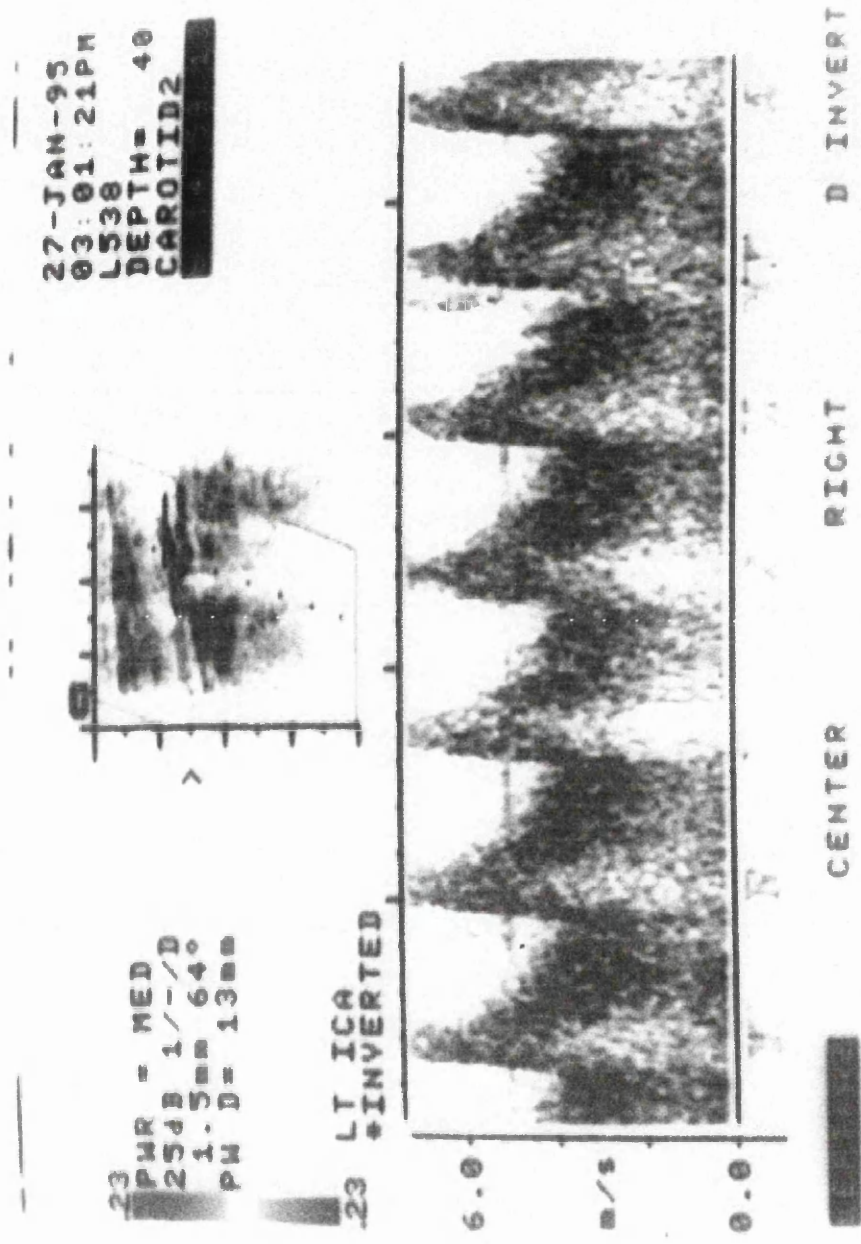


Figure 1.02.1: Relative risk of stroke by approximate mean diastolic BP among 405551 individuals without history of myocardial infarction or stroke. Solid squares represent disease risks in each category relative to risk in the whole study population. Sizes of squares are proportional to number of events in each DBP category and 95% confidence intervals for estimates of relative risk are denoted by vertical lines.

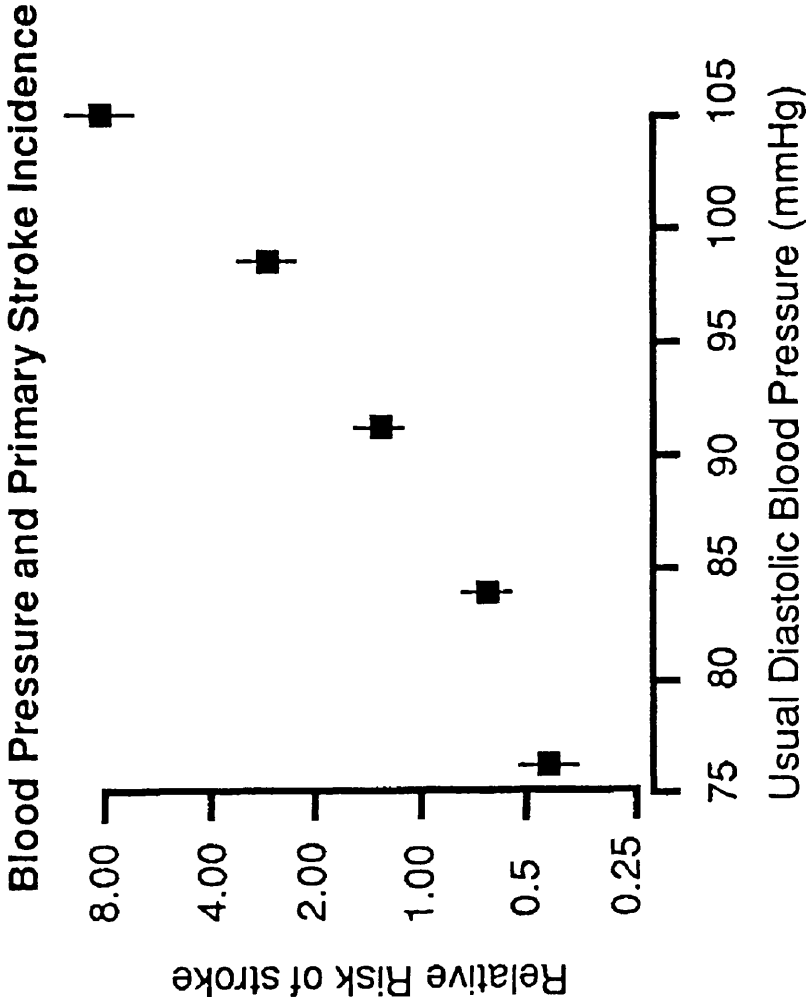


Figure 1.02.2: Relative risk of stroke in the UK TIA study by approximate mean systolic and diastolic BP (\pm SD) among 2201 individuals with a history of TIA or stroke. This study suggested a direct continuous relationship between BP and stroke incidence.

Blood Pressure and Secondary Incidence of Stroke

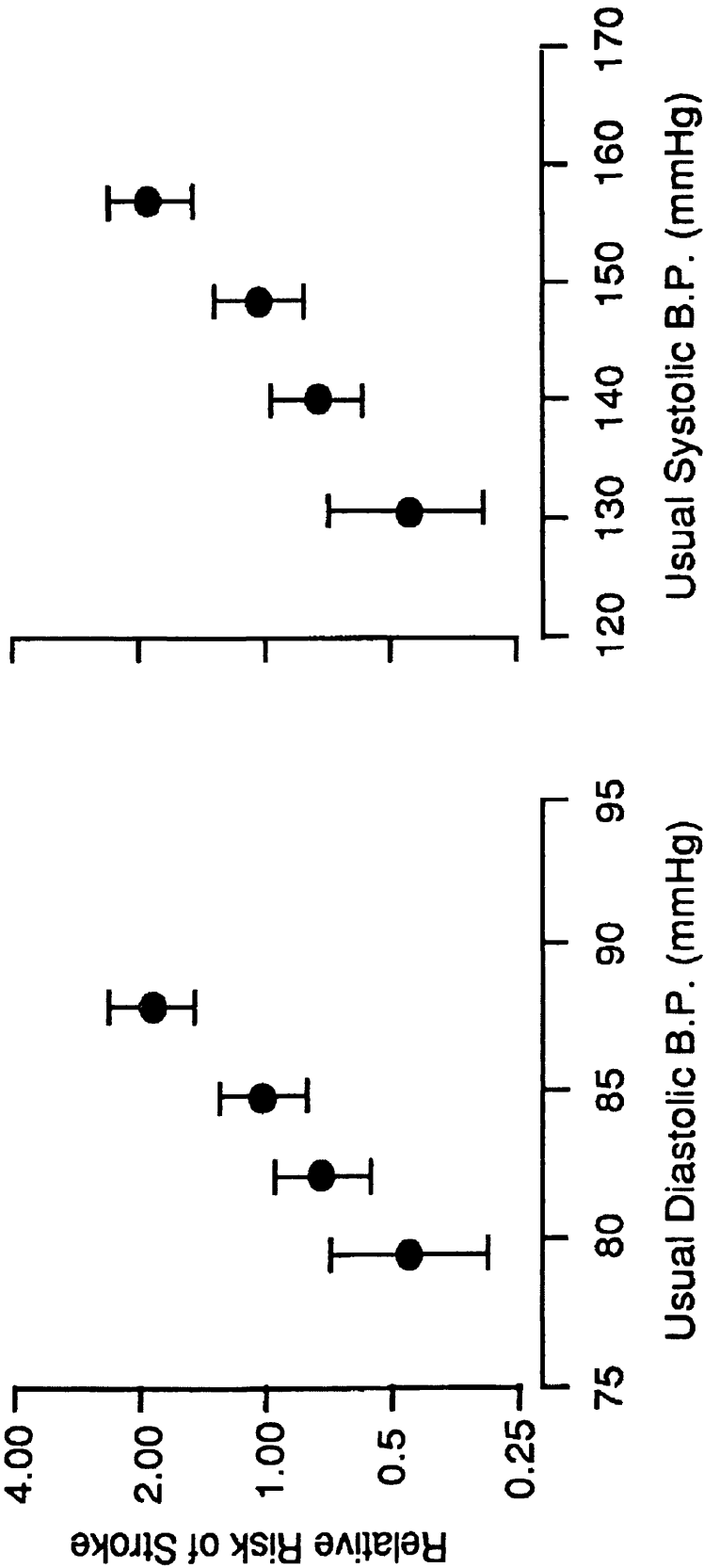


Figure 1.02.3: Stroke recurrence rates % per patient year, compared with DBP mmHg. There appeared to be an increase in the incidence of stroke recurrence in patients with DBP < 80mmHg in this Japanese study.

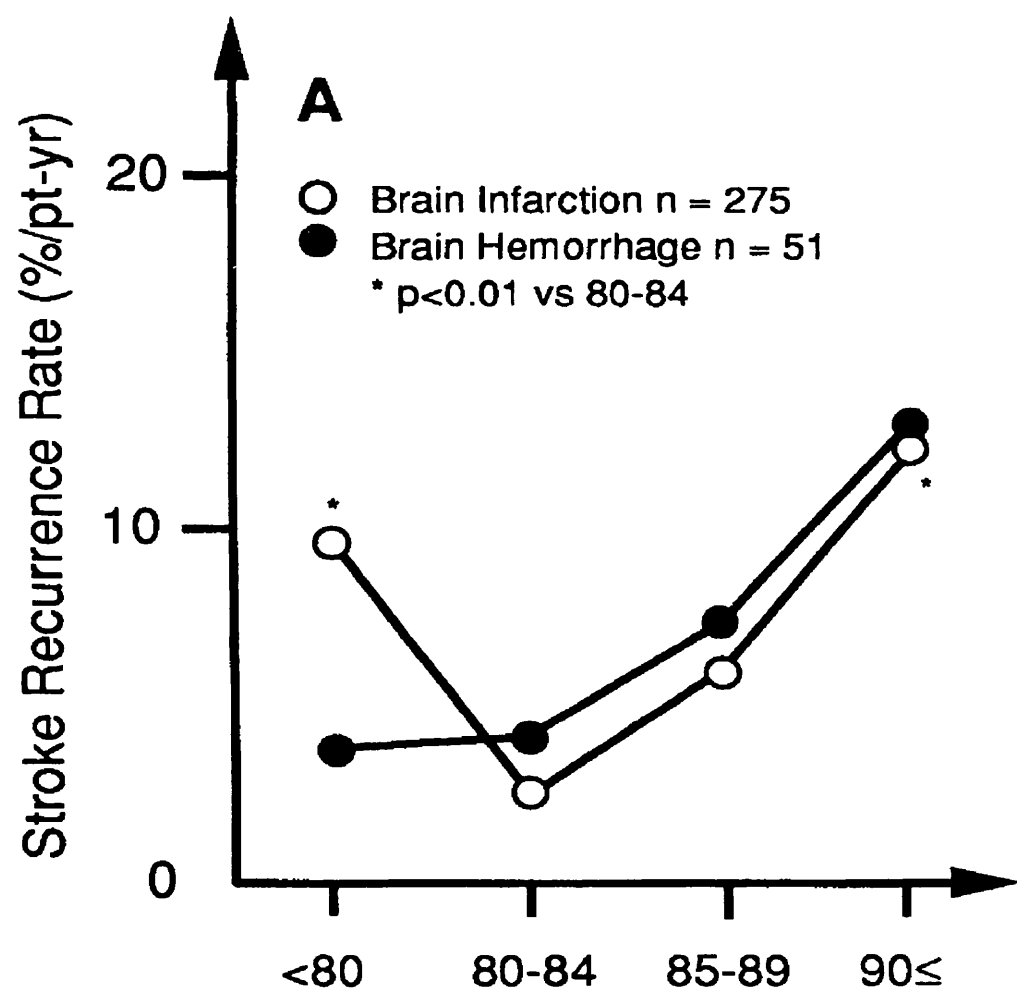


Figure 1.04.1: Combined results for stroke, coronary heart disease and death from 17 randomised, placebo controlled trials of BP lowering therapy in patients with hypertension. Mean DBP 99mmHg, mean DBP difference during follow was 5-6 mmHg, mean time from entry to avascular event or death 2-3 years.

All Randomised Trials of Antihypertensive Treatment

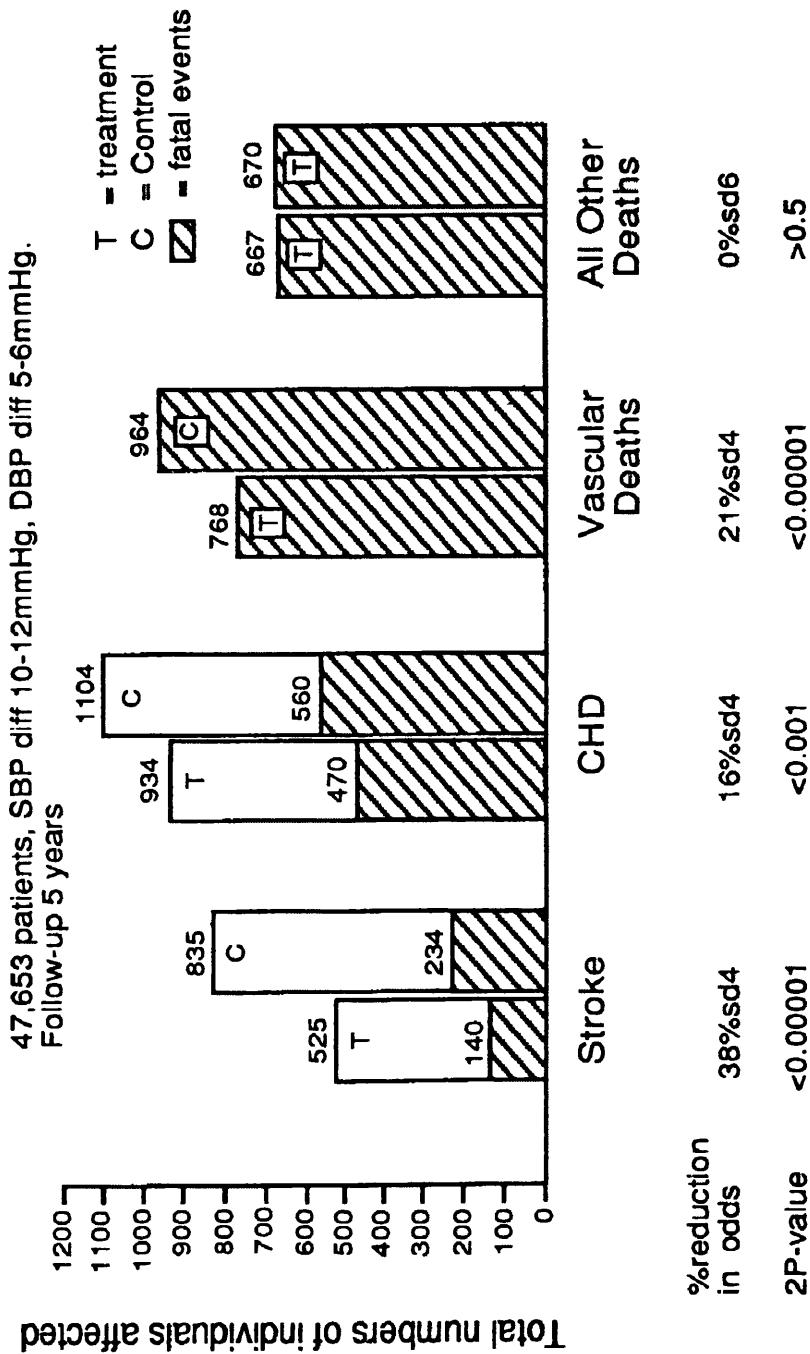


Figure 1.04.2: Fatal and non-fatal stroke rate per 100 participants on active treatment (solid line) and placebo (broken line) groups during Systolic Hypertension in the Elderly Programme.

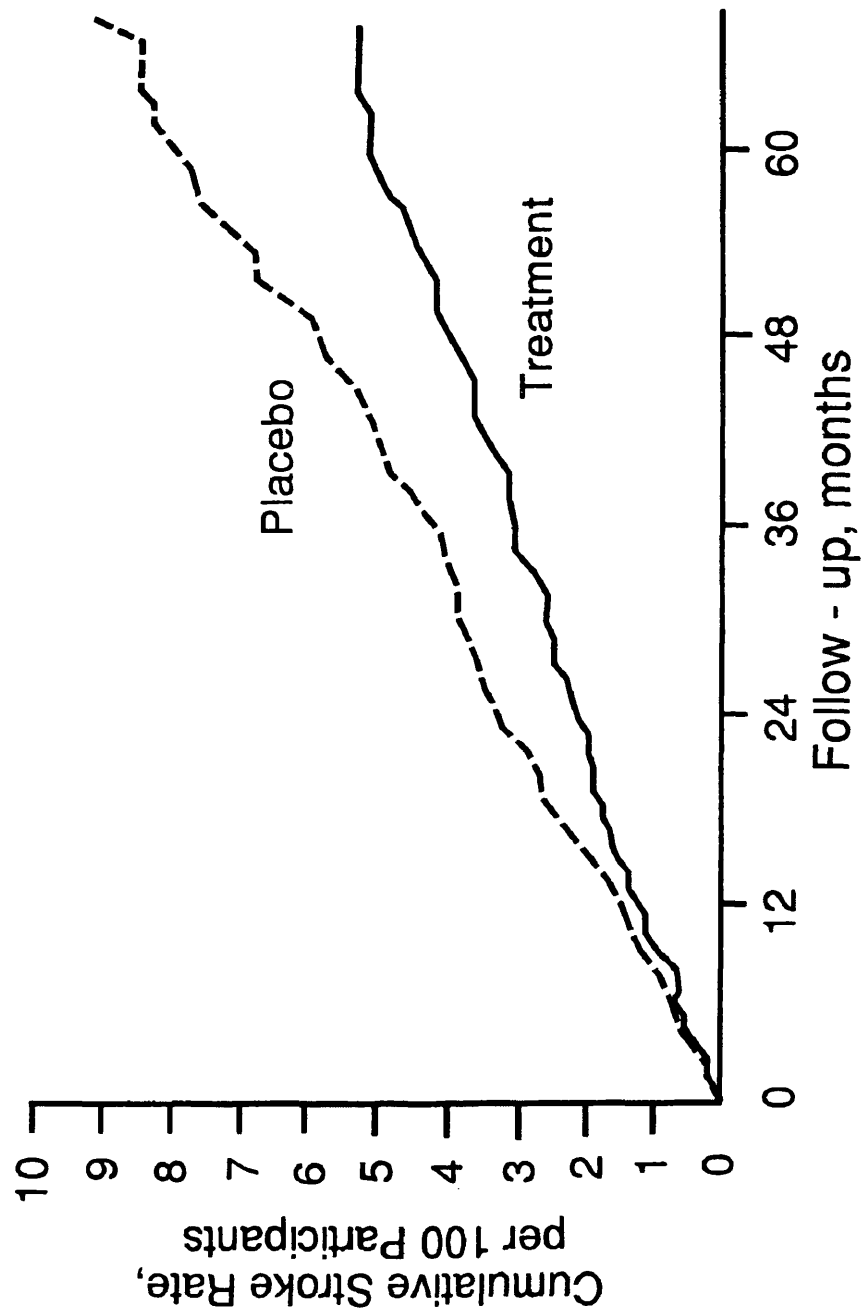


Figure 1.05.1: Results from all secondary prevention studies in patients with stroke or TIA. Carter and HSCSG studies treated hypertensives post stroke and TEST and Dutch TIA normotensives. There was a reduction in the incidence of secondary stroke but with wide confidence intervals.

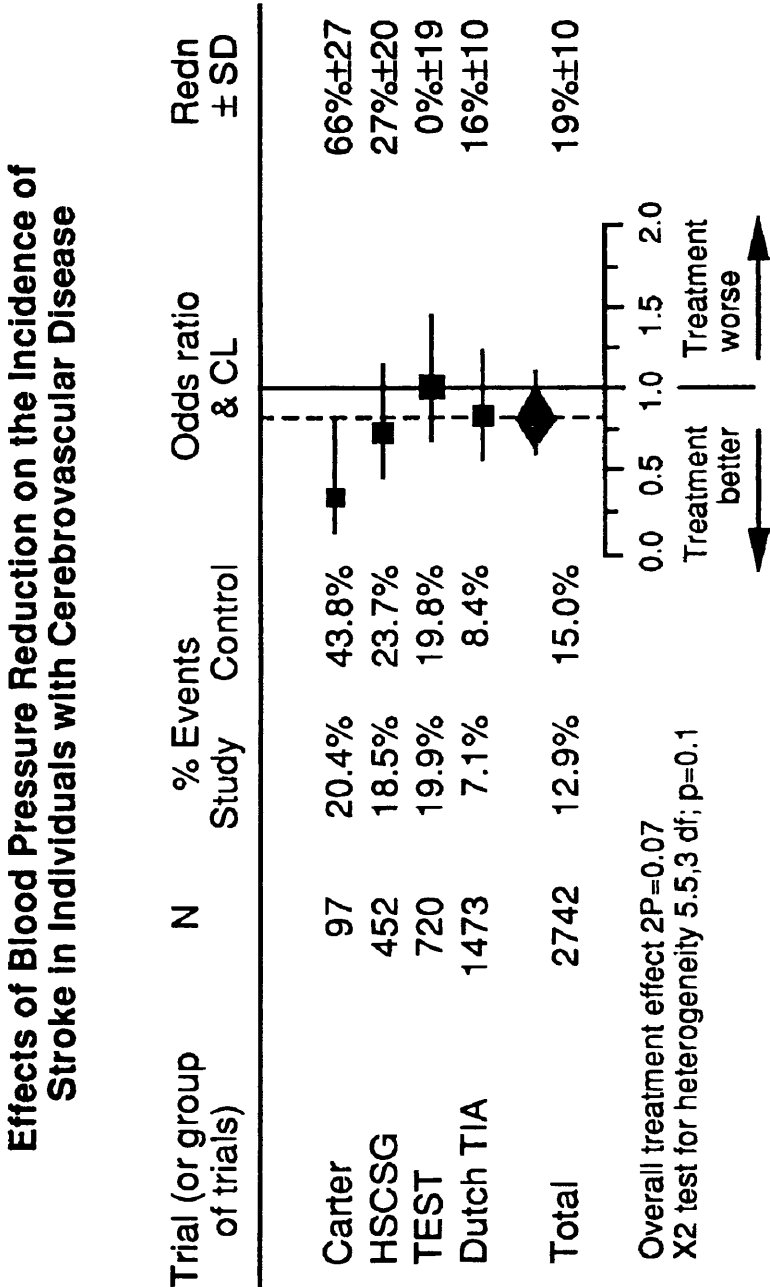


Figure 1.06.1: Systolic (a) and diastolic(b) blood pressure course in 755 stroke patients. Blood pressure falls spontaneously in patients following stroke.

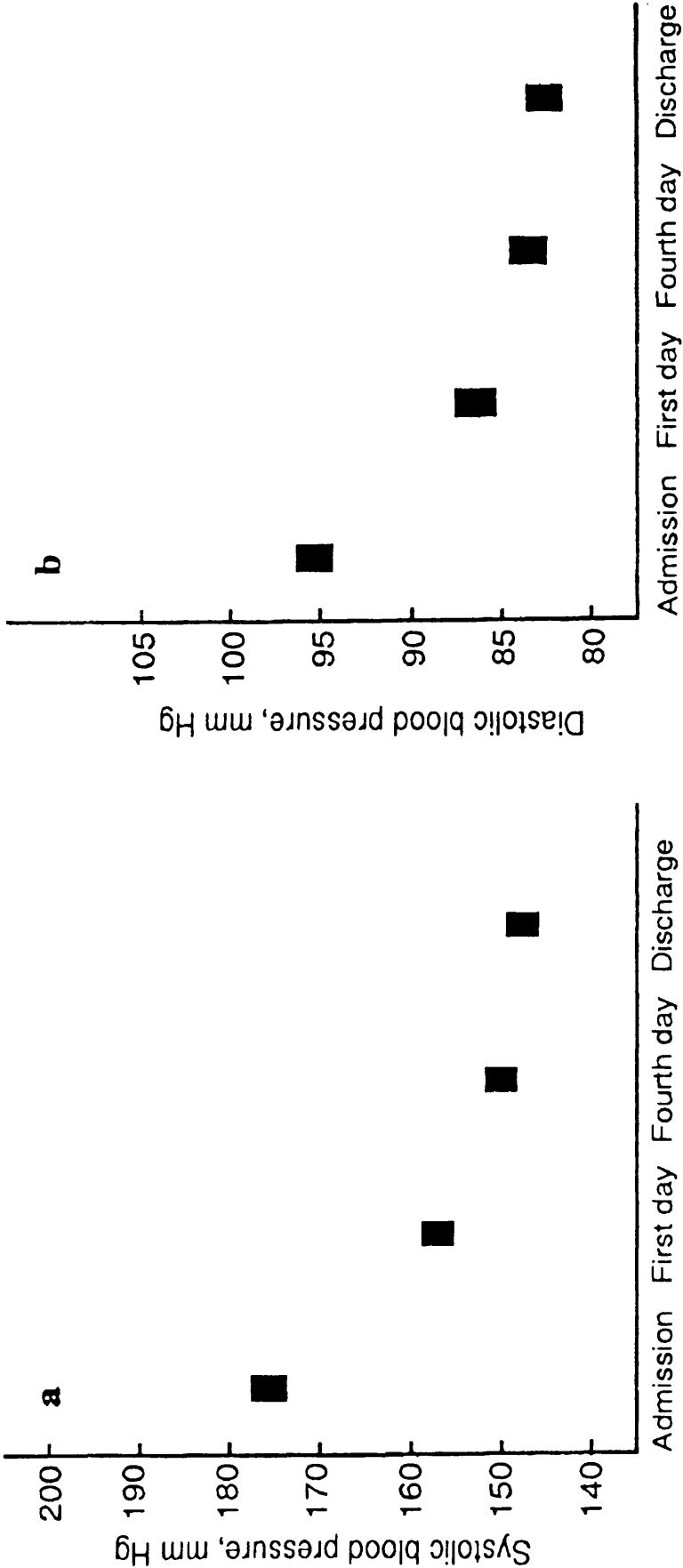


Figure 1.09.4: Mechanisms for neurotoxicity. Glutamate has a physiological role in neurotransmission, but ischaemia leads to an excess release of glutamate from presynaptic vesicles. Glia normally take up glutamate converting it to glutamine, this becomes ineffective in ischaemia. Increasing levels of glutamate activate the NMDA and AMPA receptors which facilitates depolarisation and the influx of high concentrations of calcium. Excess calcium is also released from intracellular stores. These elevated levels activate cell-damaging intracellular processes such as proteolysis, release of indible nitric oxide, phospholipases and free radical formation.

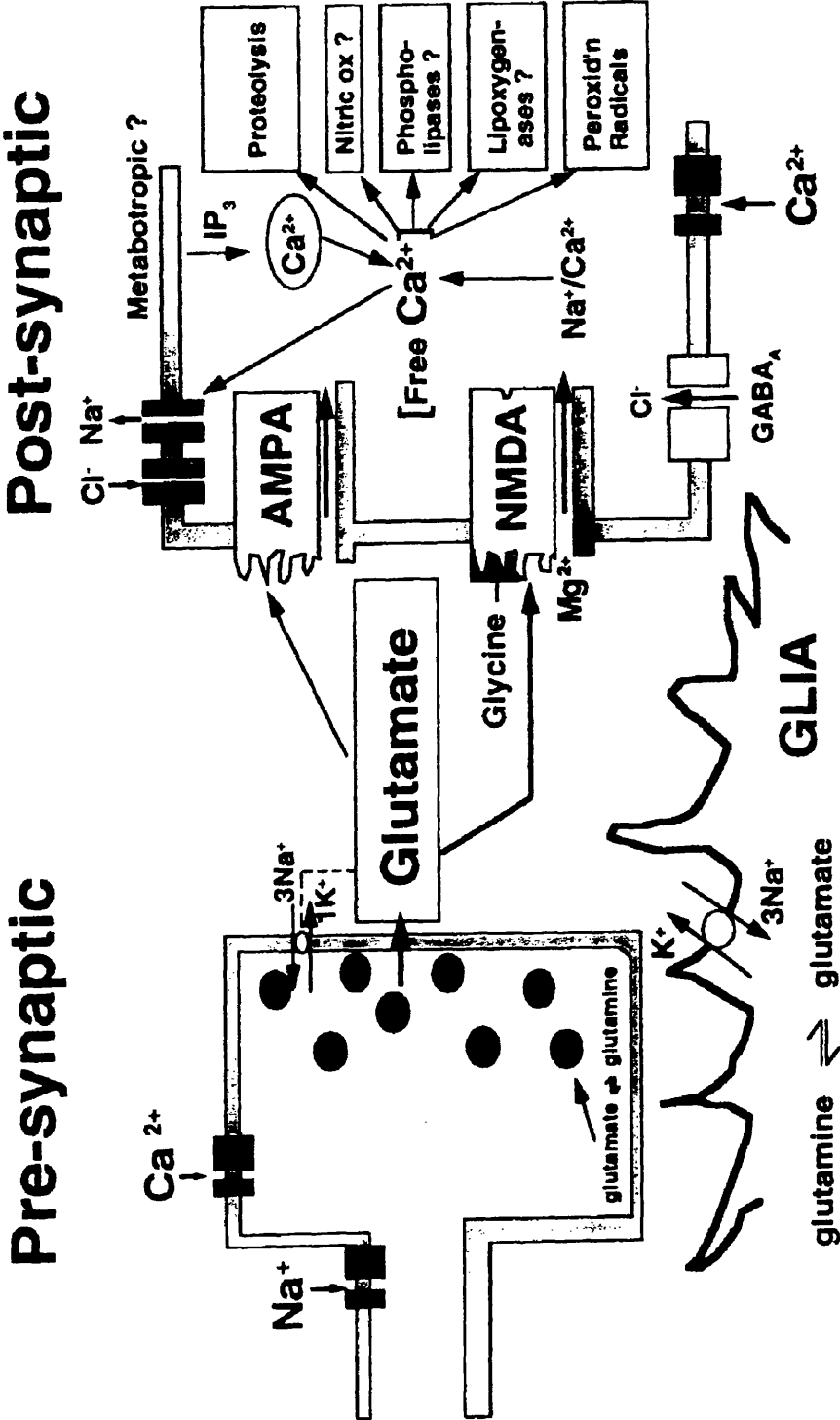


Figure 1.10.1: NINDS outcome by Barthel score at 3 months. There was a significant 30% increase in the number of patients with good clinical outcome i.e Barthel >9 5. This corresponds to full recovery or minimal disability.

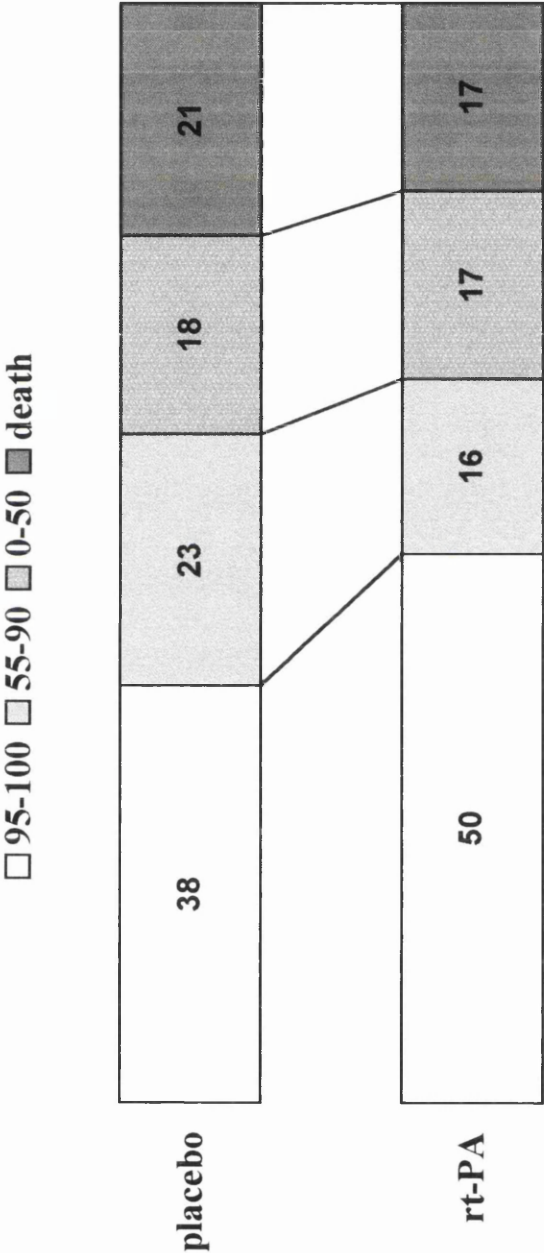


Figure 1.10.2: Trials of thrombolytic agents have consistently reported an increase in the incidence of complicating intracerebral haemorrhage in actively treated patients. This has resulted in an excess of early mortality in all studies, although this did not reach significance in the NINDS study. There was a large excess in mortality within the SK trials i.e MAST-E due to haemorrhage. There was a smaller increase in mortality due to haemorrhage within the NINDS and ECASS studies.

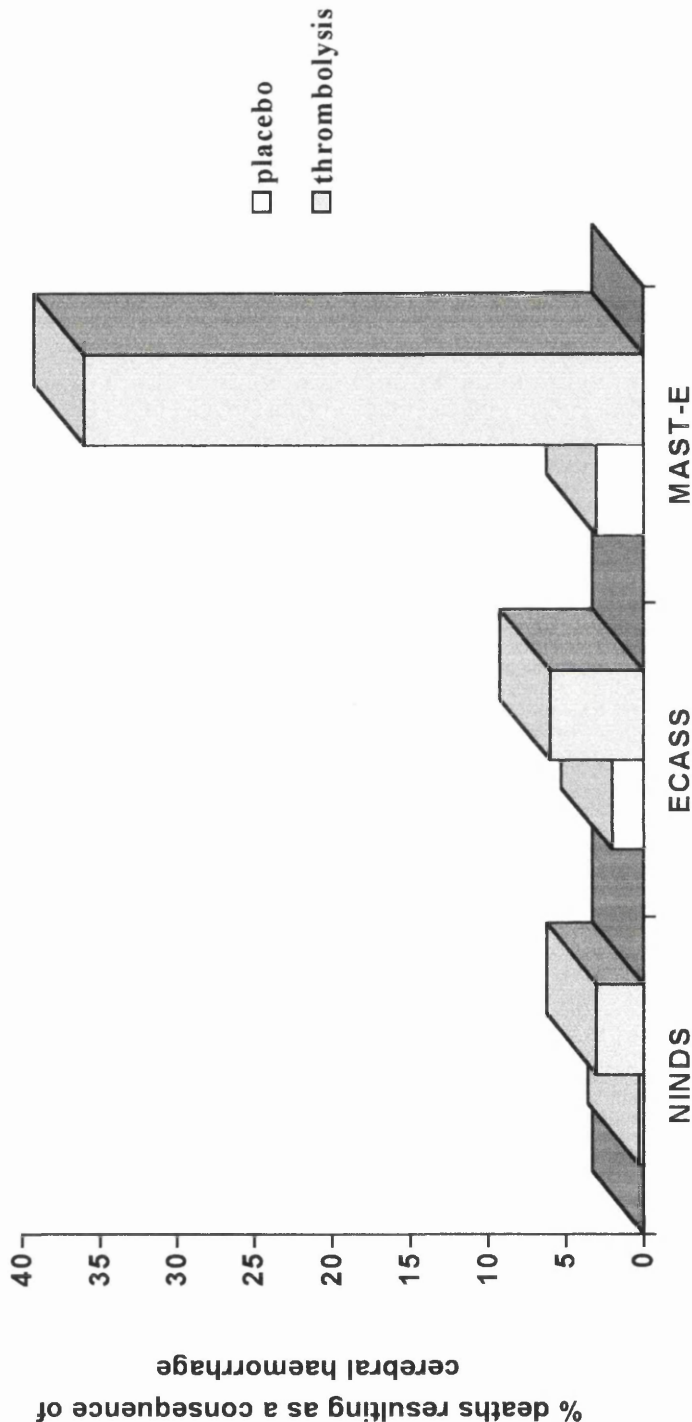


Figure 1.10.3: Incidence of complicating intracerebral haemorrhages reported in trials of thrombolytic therapy.

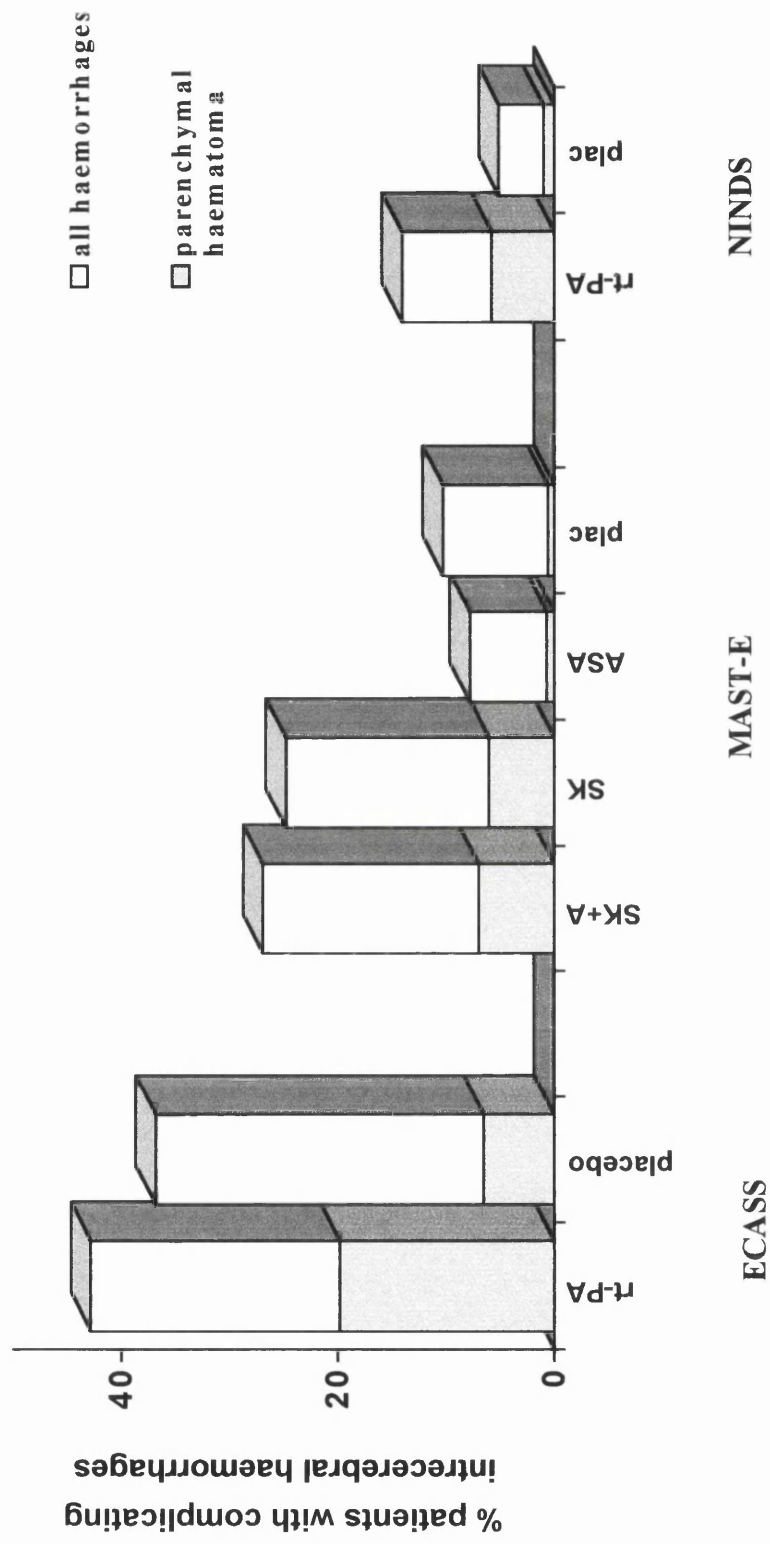


Figure 1.10.4: ECASS trial reanalysed according to NINDS study criteria (Rankin score). There were more patients with a good clinical outcome in the rt-PA treated group. This was balanced by a statistically insignificant increase in mortality.

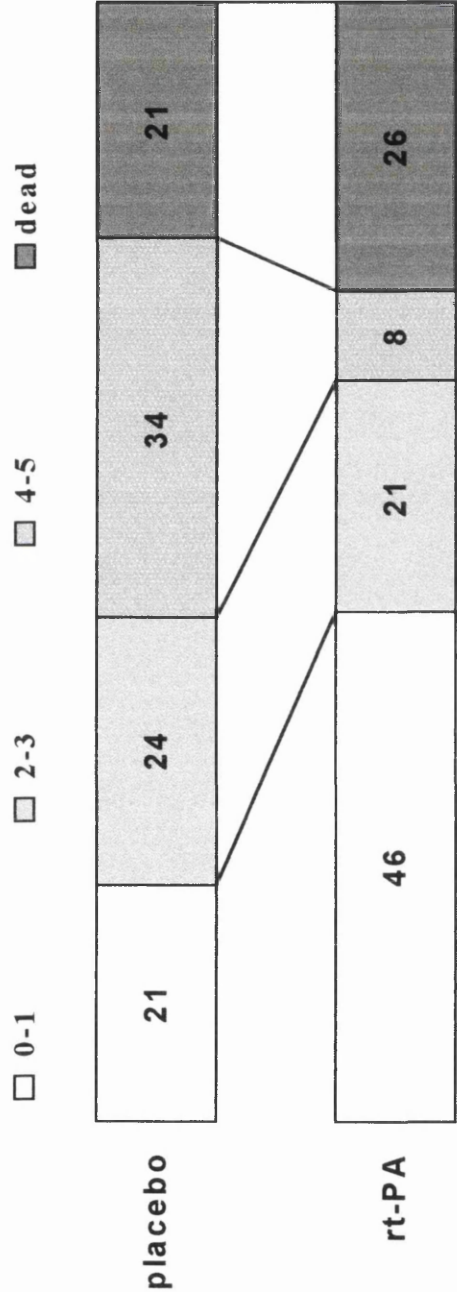
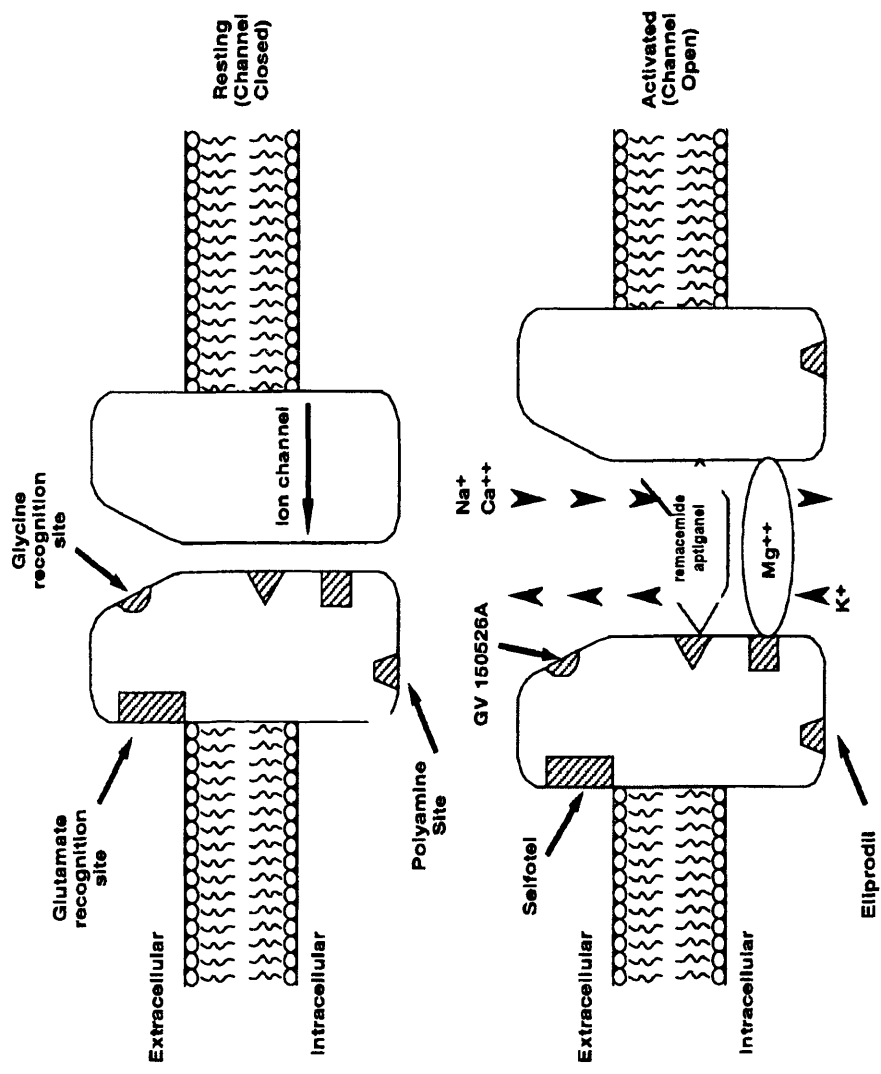


Figure 1.12.1: Schematic diagram of the NMDA receptor complex. Blockade of the NMDA receptor can be achieved at several sites. The competitive NMDA receptor antagonist selfotel acts at the agonist recognition site. Non-competitive antagonists i.e aptiganel (chapter 7) and remacemide (chapter 5) act at other distinct sites within the ion channel. The glycine binding site may be blocked by GV150526 (chapter 6). Magnesium block the activated open channel.



Chapter 2

Chapter 2

Perindopril in patients with recent cerebral infarction

2.1 Introduction

Blood pressure is an established risk factor for the primary incidence of stroke. A reduction of 5 mmHg confers a population risk reduction of stroke incidence of 30%.¹⁴ The potential benefit of antihypertensive therapy following cerebral infarction is undefined but it is likely that treatment will be of most benefit in those patients with a higher risk of future stroke i.e. those with underlying cerebrovascular disease. A definitive trial recruiting sufficient numbers of patients to demonstrate the efficacy of anti-hypertensive therapy as secondary prevention has not yet been performed but a large randomised multi-centre placebo-controlled study using perindopril and or / a thiazide diuretic (PROGRESS) will enrol between 6-8,000 patients with cerebrovascular disease and mild or moderate hypertension.^{47,48} It is hoped this study will clarify the relationship between blood pressure and the secondary incidence of stroke. Perindopril is an Angiotensin Converting Enzyme (ACE) Inhibitor with a gradual onset of action and a relatively long $t_{1/2}$ allowing once daily dosing and is less likely to cause first dose hypotension than other shorter acting preparations such as captopril or enalapril.¹⁸⁸ ACE inhibitors may be particularly suited to patients with cerebrovascular disease as they do not adversely affect cerebral blood flow.⁶⁰

Lowering blood pressure within hours of acute stroke can lead to dramatic neurological deterioration probably by reducing cerebral perfusion to the infarct zone.^{52,53} The INWEST study evaluated the effects of the calcium channel blocker nimodipine in patients within 72 hours of acute stroke. Increased mortality was associated with a reduction in blood pressure

in actively treated patients.⁵⁴ A blood pressure lowering effect was also associated with a poor clinical outcome in a phase II study of the ion channel blocker lifarizine.⁵⁵ In the first few days after acute stroke cerebral autoregulation and local cerebral perfusion is deranged and thus any change in systemic blood pressure may cause a critical reduction in local cerebral perfusion. In most cases these changes normalise within 3-4 days and cerebral autoregulation is restored.¹⁸⁹ Immediate blood pressures are often elevated in patients with acute stroke and resolve within several days of hospital admission.¹⁹⁰ It would therefore seem prudent to defer consideration of patients for anti-hypertensive therapy for at least 72 hours following hospital admission. Following this time it is still unclear which patients should go on to receive antihypertensive therapy and exactly when this should be instituted.

2.2 Materials and Methods

A double blind randomised trial design compared 15 days of oral perindopril (4 mg daily) with placebo in patients admitted to our stroke unit with a clinical and CT diagnosis of cerebral ischaemia. Patients with normal CT scans were included in the study since CT is insensitive to early signs of infarction and to small subcortical infarcts. All patients had mild to moderate hypertension (170-250 / 95-120 mmHg) as defined by two blood pressure readings within the inclusion range at least 6 hours apart within the 24 hours prior to entry into the study. BPs at the the time of drug administration were therefore not identical to screening BP readings as the latter were recorded in the hour immediately prior to drug dosing.

Patients with severe carotid disease were excluded from the study for safety and technical reasons. Patients admitted on prescribed antihypertensive therapy had treatment discontinued according to local treatment guidelines for at least 48 prior to entry into the study. Ethical approval was obtained from the West ethical committee and patients gave written informed consent to participate. Clinical and neurological assessment using the NIH stroke scale¹⁹¹ was made prior to study entry and repeated on day 15. Blood pressure was

measured semi-automatically using Marquette oscillometric equipment (Marquette Electronics Wisconsin) pre-treatment and then hourly up to 10 hours after first dosing. Blood pressure was repeated at 24 hours and at 2 weeks. Total cerebral blood flow was calculated from bilateral internal carotid artery Doppler ultrasound (Acuson 128, 5 megahertz probe Acuson, California) coupled to a wall tracker device (Wall Track System, Neurodata, Bilthoven, Netherlands). Arterial flow was calculated $= \pi \times \text{diameter}^2 \times \text{mean velocity} / 4$. Details of Doppler methods employed have been published previously.⁵⁶ MCA velocity and resistance index were measured by transcranial Doppler (Nicolet EME TC2000, 2 megahertz probe; Nicolet, Warwick, UK). Doppler recordings were undertaken pre-treatment and at 2, 4, 8 and 24 hours and again repeated at 2 weeks. An additional recording of MCA velocity was made at 6 hours. Routine safety biochemistry and hematology were collected at entry and at the conclusion of the study period. Plasma renin activity, angiotensin II activity, angiotensin converting enzyme activity and drug plasma levels were assessed at 0, 4, 6, 8, 12 and 24 hours, and at 2 weeks.

Laboratory Measurements

Plasma renin activity was measured by radio-immunoassay of generated angiotensin I (detection limit 0.54 ng /ml/hr; co-efficient of variation 6.7%). Angiotensin II was determined according to Morton and Webb¹⁹² (detection limit 2.0 pg /ml; co-efficient of variation 6.4%). Angiotensin converting enzyme was assayed by incubation of plasma/serum with the ACE substrate analogue hippuryl-histidyl-leucine. The hippuric acid produced is extracted, then quantified using high performance liquid chromatography. The limit of quantification using this assay is 0.05mMol/L. The limit of detection is 0.01 mMol/L.

Perindopril levels were assessed by the direct determination of ACE inhibitor in plasma by radioenzymatic assay using a modification of the method of Reydel-Bax et al and liquid chromatography-assisted assay for ACE in serum.¹⁹³ The active metabolite perindoprilat is

measured with a calibration range of 0.16-20 ng/ml. The limit of quantification is 0.16 ng/ml. The limit of detection is 0.1 ng/ml.

Statistical Analysis

Results were analysed using repeated measurements analysis of variance and co-variance using Statistica for Windows software version 5.0 (StatSoft Inc, Tulsa, OK, USA). With a sample size of 24 patients we expect to detect a difference in cerebral blood flow of 16% with 80% power.

2.3 Results

Tolerance and safety

A total of 28 patients were recruited to the study with 24 completing the protocol. Patients were aged between 52 and 89 years. Clinical and demographic details of patients entering the study are summarised in table 2.1 and 2.2 respectively. Four patients failed to complete the protocol: one was withdrawn following an adverse event which was not felt to be related to drug action. This consisted of transient left arm paraesthesia while undergoing carotid Doppler imaging of the right internal carotid artery 9 hours after perindopril dosing. Symptoms lasted 5 minutes and did not recur. A further patient in the perindopril group was withdrawn after only one dose when his renal function was found to be mildly impaired prior to drug treatment. Two patients receiving placebo did not complete the study: One was lost to follow up after transfer to an outlying hospital while another had inadequately documented data to allow analysis. All withdrawn patients were contacted and were well at the conclusion of the study.

Perindopril was therefore well tolerated with no serious adverse events. Biochemistry and haematology results were unremarkable. Mean NIH scores in placebo and treatment groups improved in a clinically and statistically similar manner but with no difference between the two groups (table 2.2).

Systolic, diastolic and mean blood pressures were significantly reduced in the perindopril treated patients from 2 - 24 hours after perindopril ($p < 0.004$) and remained reduced after 2 weeks treatment (perindopril $168 \pm 17 / 91 \pm 9$ at baseline to $150 \pm 21 / 79 \pm 14$ mmHg at 4 hours *cf* placebo $172 \pm 26 / 92 \pm 14$ to $173 \pm 23 / 91 \pm 13$, i.e. a placebo corrected fall of $19 / 11$ mmHg). Blood pressure changes are summarised in figures 2.1 and 2.2. There was no associated change in heart rate in either group. Despite the reduction in blood pressure there was no reduction in total internal carotid artery flow or middle cerebral artery velocity, even at the time of peak drug effect (figures 2.3 and 2.4). Internal carotid artery flow was increased at 8 hours in the perindopril receiving patients ($p < 0.004$). Neither common nor external carotid artery flow was significantly different between treatment and placebo-receiving groups. Determinations of velocity and blood vessel diameter in common, internal and external carotid vessels similarly showed no difference between perindopril and placebo groups. In addition there was no difference in the MCA resistance index (a measure of artery tone and distensibility). Renin activity and All levels were not significantly different between perindopril and placebo groups but angiotensin converting enzyme was inhibited by perindopril ($p < 0.001$) The AUC₀₋₂₄ for perindoprilat was 135 h.ng/ml (data not shown).

2.4 Discussion

Perindopril was well tolerated in patients following an acute ischaemic stroke. The study was not designed to demonstrate any long-term effect on neurological outcome but the results are reassuring as no patient suffered a drug associated neurological deterioration.

ACE inhibitors are thought to lower blood pressure without adversely affecting total cerebral blood flow. The role of angiotensin in the physiological control of the cerebral circulation has not been adequately defined. The configuration of the ACE gene may be important in the generation of accelerated atherosclerosis in the coronary and cerebral circulations, although there is conflicting evidence that ACE genotype is relevant in the development of cerebrovascular disease. Angiotensin 2 (AT₂) receptors regulate cerebral blood flow in rats. Large cerebral arteries containing AT₂ receptors ameliorate increases in blood flow in response to a rise in blood pressure.¹⁹⁴ Treatment of hypertensive animals with ACE inhibitors resets cerebral autoregulation at a lower level but this effect may be shared with other antihypertensive agents. In hypertensive humans without a history of stroke, captopril increases cerebral blood flow, measured by a single positron emission computed tomography scanning (SPECT) radionuclide Xe -133 technique, with an inverse correlation between BP fall and mean cerebral blood flow.¹⁹⁵

Two single dose studies in healthy volunteers by Demolis assessing blood flow with carotid and transcranial Doppler following ACE inhibitor administration demonstrated similar results to our study, with BP effectively lowered and an increase in bilateral common carotid artery flow.^{196,197} Middle cerebral artery flow velocity was unchanged but there was an increase in cerebral vascular resistance index suggesting vasoconstriction in the cerebral arterioles.¹⁹⁶

Hypertensive stroke patients have only been assessed in two uncontrolled studies (each recruiting 12 patients). Both studies used SPECT scanning and a Xe 133 inhalation technique. In one study drug effectively lowered BP and increased cerebral blood flow to both hemispheres¹⁹⁷ while in the other study a fall in blood pressure was not associated with a significant blood flow effect.⁶⁰

Doppler data support the hypothesis that perindopril does not adversely affect cerebral blood flow or alter cerebral hemodynamics in a clinically significant way. The results, however cannot be considered relevant to all patients with severe carotid disease. It is conceivable that the presence of hemodynamically significant carotid lesions may lead to a reduction in cerebral perfusion distal to a site of stenosis following the lowering of systemic blood pressure. This may be particularly relevant in the hours and days immediately following acute stroke where cerebral autoregulation is deranged and consequently perfusion is directly dependent on systemic blood pressure levels. We did not consider it ethical to treat patients prior to 48 hours of onset of stroke symptoms as there is good trial evidence that lowering blood pressure at this time results in adverse outcome.^{54,55} Further research is required to assess whether these patients are indeed more prone to neurological deterioration following BP reduction before treatment guidelines can be advised. It is also possible that while total internal carotid artery flow is preserved, local ischaemic areas may become increasingly compromised as a result of a reduction in blood pressure. Other forms of brain imaging techniques such as SPECT (Single Photon Emission Computed Tomography) or PET (Positron Emission Tomography) scanning may give further information on the effects of blood pressure lowering treatment on regional perfusion particularly in the area surrounding the cerebral infarct.

Our data suggest that starting perindopril treatment within 2 and 7 days of the onset of cerebral ischaemia can successfully and safely lower blood pressure without adversely affecting total cerebral blood flow in patients without severe carotid stenosis.

Table 2.1: Clinical details of patients at entry to study. Clinical stroke classification: LACI - lacunar infarction. TACI - total anterior circulatory infarction. PACI - partial anterior circulatory infarction. POCI - posterior circulatory infarction. NB. All CT lesions consistent with infarction.

number	drug	patient	prior cva	prior BP	BP Rx	CT	Clinical	Doppler	Age	Sex
1	0 MAH	N	N	N	N	N	right LACI	N	58	M
2	0 MUL	N	N	N	N	N	left LACI	mild left stenosis	70	M
3	1 LAI	N	Y	atenolol, bendrofluazide	N	N	left POCI	N	81	F
4	1 TAI	N	Y	N	N	left subcortical	left LACI TIAN	N	58	M
5	1 BAI	N	Y	atenolol	N	left subcortical	left PACI	N	79	F
6	1 DOR	Y	Y	atenolol, bumetanide, minoxid	N	right subcortical old	POCI	N	63	M
7	0 MUR	N	N	N	N	left subcortical	left PACI	N	71	M
8	0 HAR	N	Y	bendrofluazide	N	right subcortical	right LACI	N	81	F
9	0 NEA	N	Y	bendrofluazide	N	left subcortical	right LACI	N	68	F
10	0 MCI	N	N	N	N	N	left PACI	N	64	F
11	1 CRO	N	N	N	N	old left cortical	right LACI	N	65	M
12	1 WHY	N	Y	metoprolol	N	N	POCI	left stenosis	52	M
13	0 KID	N	Y	N	N	new left cortical	left PACI	N	83	M
14	0 GUI	N	Y	nifedipine retard	N	N	left LACI	N	58	M
15	1 HOW	N	Y	neonaclex-K	N	new right cortical, multiple old infarcts	right LACI	mild right stenosis	86	M
16	1 DUC	N	N	N	N	right subcortical	right LACI	mild right stenosis	80	F
17	1 WAR	N	Y	nifedipine	N	old left subcortical, bilateral old brainstem and cerebellar infarcts	POCI	N	75	M
18	0 MCF	N	Y	bendrofluazide	N	left subcortical	left LACI	N	58	F
19	0 KEL	N	N	N	N	multiple cortical and subcortical bilateral infarcts	right LACI	N	78	M
20	1 CRA	N	Y	bendrofluazide	N	right subcortical	right LACI	N	85	F
21	1 MUL	N	N	N	N	right subcortical	right PACI	N	65	M
22	1 MEL	Y	Y	tenoretic	N	right cortical/subcortical	right LACI	N	64	F
23	0 MOO	Y	Y	amlodipine	N	right cortical	right PACI	N	76	F
24	0 WAT	N	Y	N	N	right cortical	right LACI	N	70	M
25	0 MAR	N	N	N	N	right subcortical	right LACI	N	57	M
26	0 HAR	N	Y	N	N	right subcortical	right LACI	N	89	F
27	1 PRI	N	Y	N	N	old bilateral subcortical	right LACI	N	62	M
28	1 NIC	Y	N	N	N	old left subcortical	left LACI	N	66	M

Table 2.2: Demographic details of patients at entry to the study.

SBP - Systolic blood pressure, DBP - Diastolic blood pressure, NIH 1- NIH Stroke scale score at entry to study. NIH 2 - NIH Stroke score at end of 15 day period.

	Treatment	Age	Height cm	Weight lb	SBP mmHg	DBP mmHg	Days since cva	NIH 1	NIH 2
Placebo	Mean	70.5	162.3	153	172.5	91.3	2.7	3.8	2.4
n = 12	Std. Err.	3.0	3.1	8.0	7.4	4.2	0.3	2.8	2.6
Perindopril	Mean	67.4	167.8	145.0	170.8	94	3.2	3.1	1.6
n = 12	Std. Err.	2.7	2.6	14.0	5.2	3.6	0.4	3.3	1.1

Figure 2.1: Systolic blood pressure (\pm SE of the mean) v. time in patients receiving perindopril or placebo. Systolic blood pressures were significantly reduced in the actively treated group from 2 - 24 hours after perindopril ($p < 0.004$) and remained reduced after 2 weeks treatment.

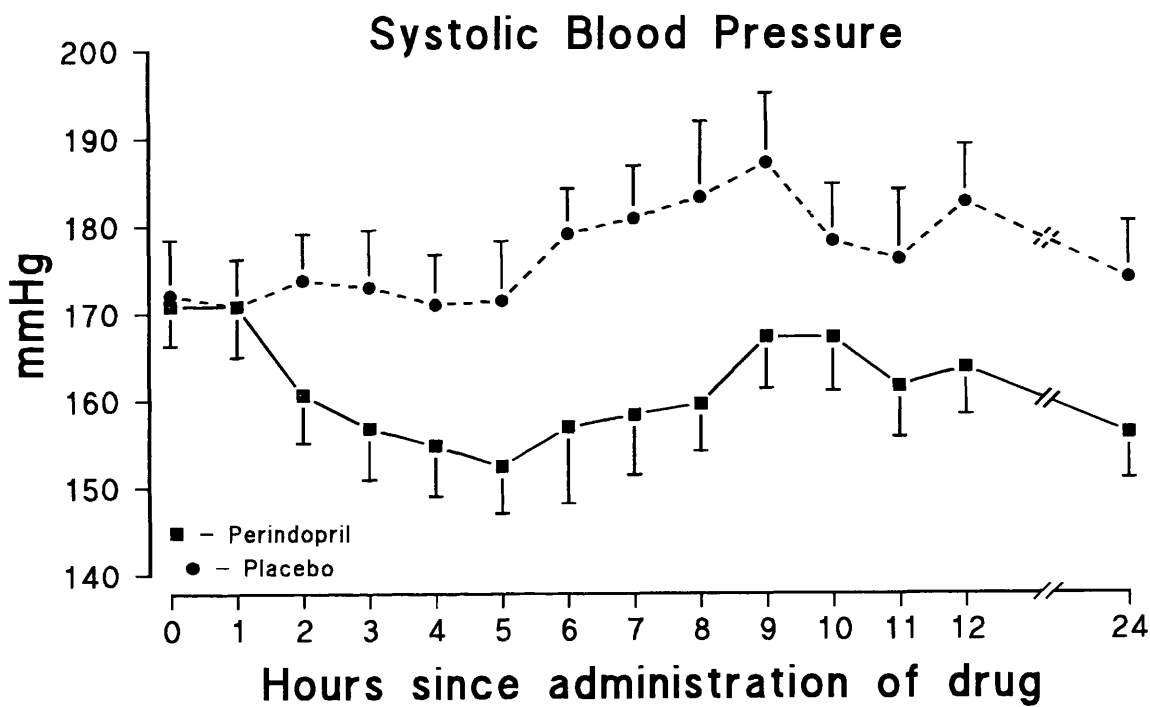


Figure 2.2: Diastolic blood pressure v. time in patients receiving perindopril or placebo. Diastolic blood pressure (\pm SE of the mean) v. time in patients receiving perindopril or placebo. Diastolic blood pressures were significantly reduced in the actively treated group from 2 - 24 hours after perindopril ($p < 0.004$) and remained reduced after 2 weeks treatment.

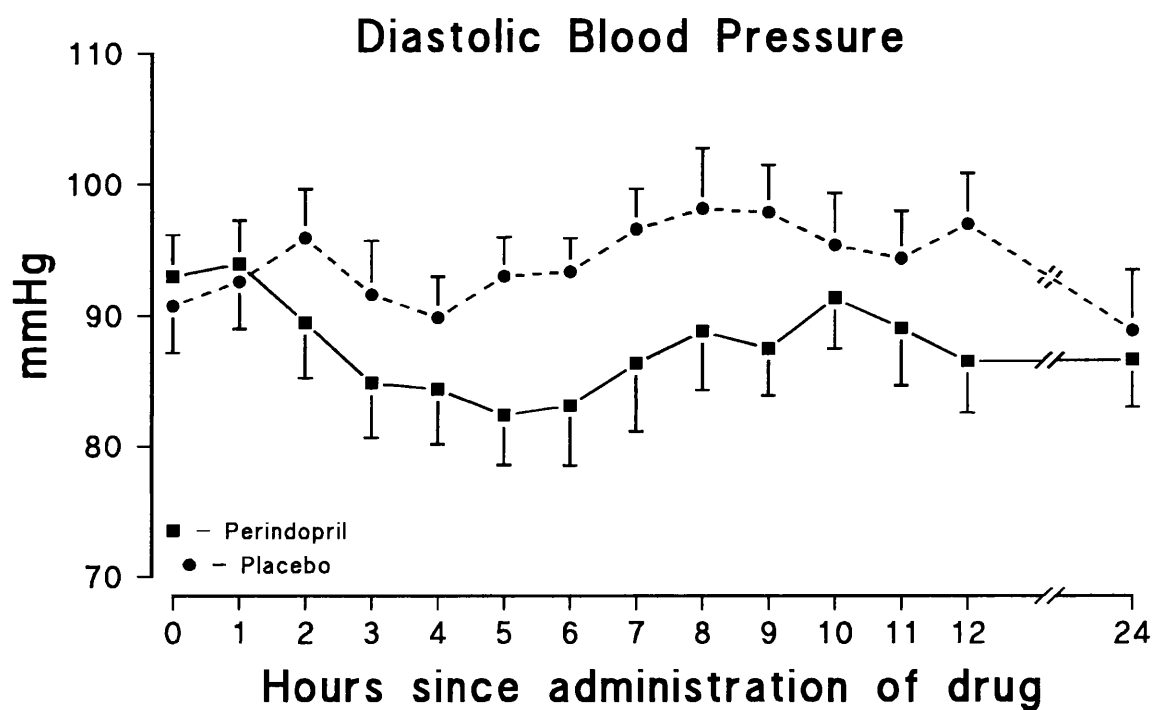


Figure 2.3: ICA flow (\pm SE mean) vs time in patients receiving perindopril or placebo ICA (internal carotid artery flow rate mL/minute) vs time in patients receiving perindopril vs placebo. Increase in flow in the actively treated group at eight hours ($p<0.004$)but no significant changes at other time points.

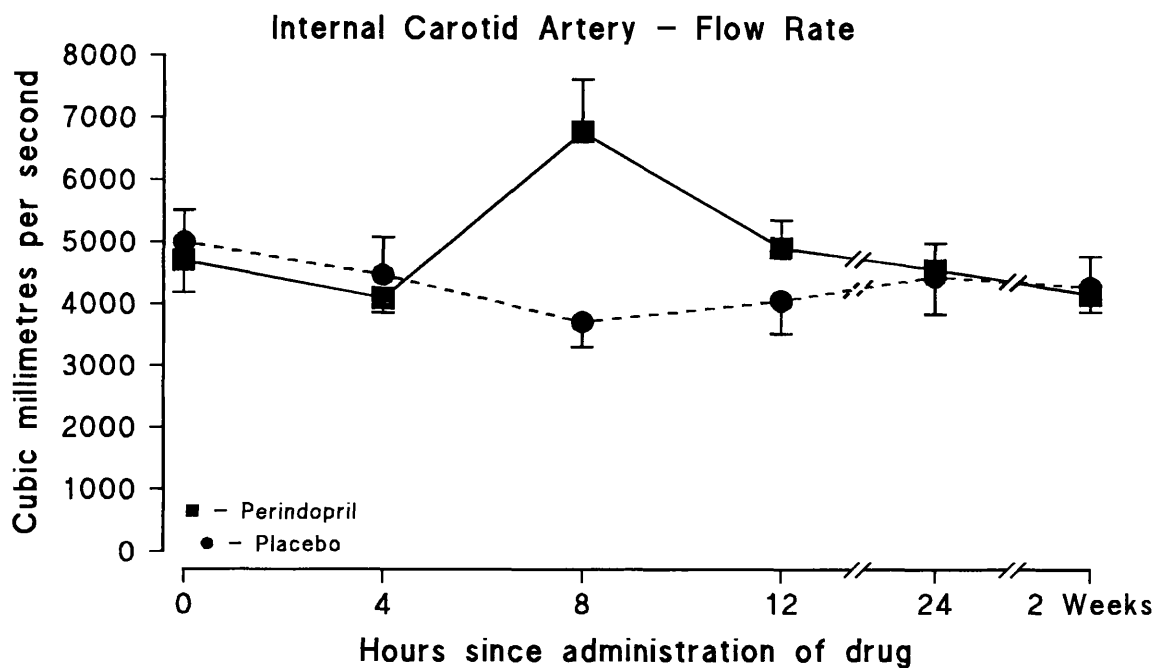
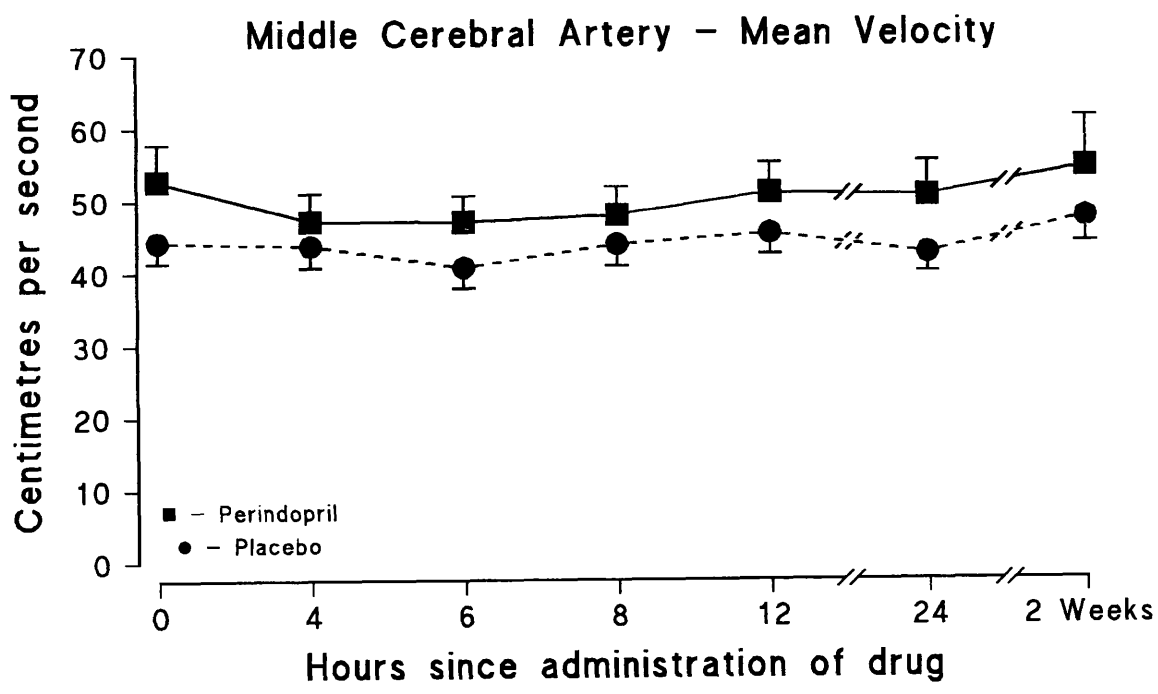


Figure 2.4: MCA (middle cerebral artery) mean velocity cm/s (\pm SE mean) vs time in patients receiving perindopril or placebo. There was no significant changes over time and no difference between placebo and perindopril receiving groups.



Chapter 3

Chapter 3

Influence of cholesterol on survival after stroke

3.1 Introduction

The association between total serum cholesterol and coronary artery disease is well established but the relationship between stroke and cholesterol remains less clear. Large epidemiological studies in Japanese^{198,199} and Japanese Americans²⁰⁰ failed to associate cerebral infarction with raised cholesterol but found an inverse relationship with the incidence of intracerebral haemorrhage. The MRFIT study examined middle aged men in the US and noted a positive association between raised cholesterol and ischaemic stroke and a negative association with haemorrhagic events.²⁰¹

Meta-analysis of the prospective follow up of 450,000 patients within 45 separate cohorts over an average follow-up period of 16 years detected no relationship between cholesterol and the overall incidence of stroke.²⁰² In most cohorts however there was no differentiation between stroke type i.e. cerebral infarction or intracerebral haemorrhage and competing associations with infarction and a negative association with haemorrhage could not be excluded.

A post mortem study in Japanese stroke patients did suggest an association between low cholesterol and cerebral haemorrhage and between high cholesterol and large carotid vessel atherothrombotic infarction. There was no association between subcortical lacunar events and raised cholesterol.²⁰³

Meta-analyses of published trials of lipid lowering treatment and incidence of stroke are fraught with potential bias and pitfalls but not all studies support the hypothesis that lowering cholesterol effectively reduces the incidence of stroke. In the WOSCOPS (West of Scotland Coronary Prevention Study) pravastatin successfully reduced cholesterol and the primary incidence of coronary events but not stroke in a middle aged population²⁰⁴. The CARE study was a large placebo controlled study recruiting 4159 patients study investigating the effect of pravastatin treatment for 5 years on patients with previous myocardial infarction and average cholesterol. The results suggested a significant reduction in total mortality and a reduction in the incidence of stroke by 31% ($P= 0.03$)²⁰⁵. Patients in the simvastatin receiving group of the 4S study (lipid lowering as secondary prevention of coronary heart disease) also had a significantly reduced number of cerebrovascular events²⁰⁶.

Lipid lowering therapy particularly with statins have been shown to reverse carotid artery atheroma formation with an associated reduction in cerebrovascular events. Lovastatin and pravastatin have both been shown to reversed intimal medial thickening in the carotid arteries.^{207, 208}

There are a number of possible explanations as to why a reduction in stroke incidence has not been consistently reported in studies of lipid lowering therapy. Many large trials of cholesterol lowering treatment were designed to detect a reduction in coronary deaths in middle aged patients with and without coronary artery disease. Coronary events are 10 times more common than stroke in this age group of patient and as a result few stroke endpoints per study were reported. The few studies that have been conducted in elderly patients do however suggest a reduction in stroke incidence with lipid lowering treatment. It is also likely that cholesterol influences certain subtypes of stroke and any effect is likely to be diluted if strokes are not divided into the appropriate diagnostic grouping. Distinction between cerebral

haemorrhage and infarction is absolutely essential as available evidence suggests there is a positive association with cerebral infarction and a reverse relationship with haemorrhage.

As yet there are no published data regarding the effect of cholesterol on survival or prognosis following stroke. We considered the hypothesis that hypercholesterolaemia would predispose to a poorer outcome and an increased mortality from stroke but infact our analysis demonstrated a counter-intuitive 'protective' effect of relatively higher cholesterol.

3.2 Subjects and methods

The admission criteria and protocol of the acute stroke unit of the Western Infirmary, Glasgow, are described in detail elsewhere.²⁰⁹ Briefly, all patients within a well-defined geographical region suffering a new focal or global neurological deficit are admitted, regardless of age or severity of neurological deficit. CT or MRI is performed routinely within 72 hours of admission. Total serum cholesterol is measured on a fasting sample on the morning following admission. Details of each patient's risk factors, presenting complaints, neurological examination, results of investigations, and final diagnosis are prospectively recorded and transferred to a computerised database. The patients included in this study represent a series of consecutive admissions to our unit. Patients whose symptoms were found to be caused by a condition other than stroke are excluded from the analysis.

Total serum cholesterol was measured by a standard cholesterol oxidase method. Subtype of stroke (primary intracerebral haemorrhage [PICH] or infarction) was diagnosed from the early CT or MR scan. Stroke was classified according to the system used by the Oxfordshire Community Stroke Project.³ This describes patients as having total anterior circulation infarction (TACI), partial anterior circulation infarction (PACI), posterior circulation infarction (POCI) or lacunar infarction (LACI).

Outcome follow-up was by record linkage²¹⁰ to death records from the Registrar General of Scotland, and to hospital discharge records to obtain information on medical events after stroke. This technique has been validated previously in an epidemiological study of hypertension²¹¹ and has also been used for endpoint monitoring in a large clinical trial.²¹² The method of record linkage is a reliable one; however, admissions to private hospitals or institutions outwith Scotland are not detected. Outcome was categorised as alive at home, alive in care, or dead at 2, 3, 6 and 12 months after stroke; only the 3-month data were used in our study, with good outcome defined as alive at home *versus* alive in care or dead. This subdivision at 3 months is a marker for 3-month functional outcome, an endpoint commonly used in trials of therapeutic agents in stroke.

We assessed the effect of serum cholesterol on survival using Cox's proportional hazard model²¹³ in which we controlled for known confounding factors affecting outcome (stroke type, i.e. haemorrhage or infarction; symptom duration, i.e. transient ischaemic attack (TIA) or reversible neurological deficit *versus* sustained stroke; Oxfordshire classification of stroke; plasma glucose concentration; and age). Since stroke subtype may not satisfy the proportionality assumption of the Cox proportional hazard model, we stratified the analysis into 5 groups for sub-type of stroke and Oxfordshire category (PICH, TACI, PACI, POCI and LACI). Age and serum cholesterol were entered as continuous independent variables. Plasma glucose did not satisfy the proportionality assumption and was entered as a dichotomous variable (≤ 8 mmol/L *versus* > 8 mmol/L). Estimates of survival for 1000 days in example cases are provided from the Cox proportional hazard model. The model predictions were checked by comparison with Kaplan-Meier survivorship functions for selected subgroups of patients. Dichotomous outcome at 3 months was assessed by logistic regression, using the same independent variables as above. We performed the statistical analysis using Statistica for Windows Version 5.0 (StatSoft Inc, Tulsa, OK, USA) on a PC. Statistical significance is declared at $P < 0.05$. Normally distributed data are expressed as mean \pm SD.

3.3 Results

One thousand one hundred and sixty five patients were included in this study, after exclusion of 227 admissions in 1990 and early 1991 for whom Oxfordshire classification and/or CT scan results were unavailable. Of these 1165 patients, 102 had suffered a transient ischaemic attack and 43 a reversible neurological deficit; the duration of symptoms was not recorded in 9. Thus, complete data were available for 1011 patients with acute stroke. The average age was 69.9 ± 12.4 years (range 23-94); the average total serum cholesterol concentration was 5.91 ± 1.43 mmol/L; and plasma glucose was 7.3 ± 2.7 mmol/L. In survivors, the mean follow up was 895 days, range 105-2032, with total mortality during the follow up period of 39.4%: median survival could not be estimated but the 25th percentile was 254 days. There were 109 patients with primary intracerebral haemorrhage (1000 day survival 38%), 198 with TACI (1000 day survival 38%), 326 with PACI (1000 day survival 63%), 91 with POCI (1000 day survival 71%) and 287 with LACI (1000 day survival 64%). Mortality was also significantly influenced by the presence of hyperglycaemia (plasma glucose > 8 mmol/L): 1000 day survival was 60% if normoglycaemic *versus* 43% if hyperglycaemic. Age significantly influenced outcome, with 1000 day survival of 71% in patients aged ≤ 69 years and 46% for the older patients. Serum cholesterol concentration did not correlate with plasma glucose ($r^2 = 0.002$) or with age ($r^2 = -0.004$).

Figure 3.1 shows the Kaplan-Meier cumulative survival curves for patients with above or below average cholesterol concentrations in our population. Within the Cox proportional hazard model after adjustment for stroke type and Oxfordshire group, higher plasma glucose, lower serum cholesterol and higher age were independent predictors of mortality after stroke (table 3.1). The relative hazard was 8% lower for each 1 mmol/L rise in serum cholesterol concentration. The effect of cholesterol appeared similar within each stroke subgroup,

though it reached statistical significance only within the larger subgroups: patients with total or partial anterior circulation infarcts (table 3.2).

Within logistic regression, when outcome at 3 months was coded as good (alive at home) or bad (alive in care, or dead) and after adjustment for age, plasma glucose, stroke type and Oxfordshire group, increasing levels of serum cholesterol were still associated with better outcome (P=0.024).

Examples of the predicted 1000-day survival for patients with low or high cholesterol at different ages are shown in table 3. 3.

Table 3.1: Cox proportional hazard model for mortality after stroke in 1011 patients, adjusted for stroke type and Oxfordshire classification (PICH, TACI, PACI, POCI or LACI): $\chi^2 = 70.0$, $df = 3$, $P<0.00001$.

	relative hazard	95% confidence intervals	P value
plasma glucose (>8 versus ≤8 mmol/L)	1.60	1.30, 2.00	0.0002
serum cholesterol (per mmol/L)	0.92	0.84, 0.98	0.017
age (per year)	1.028	1.018, 1.038	0.00001

Table 3.2: Relative hazard associated with serum cholesterol within each stroke subtype/Oxfordshire category.

Group	relative hazard	95% confidence intervals	n
PICH	0.97	0.78, 1.20	109
TACI	0.88	0.78, 1.03	198
PACI	0.88	0.76, 1.00	326
POCI	0.99	0.71, 1.38	91
LACI	0.95	0.82, 1.12	287

Table 3.3: Predictions from Cox proportional hazard model according to age and total serum cholesterol concentration, after adjustment for stroke subtype and Oxfordshire classification.

age (years)	serum cholesterol (mmol/L)	1000-day survival (%)
50	5.2	73
50	7.5	78
75	5.2	51
75	7.5	59

3.4 Discussion

Our data suggest that the level of cholesterol independently influences survival in patients with acute stroke. Higher levels of cholesterol were associated with improved survival. The effect appears greatest in elderly patients e.g. 1000 day survival would be 51% for a 75 year old patient and a cholesterol of 5.2 mM, and 59% if the cholesterol is 7.5mM, an absolute risk reduction of 8% and a relative risk reduction of 16%. Cholesterol did not correlate with either blood sugar or age. The prognostic effect was robust following adjustment for known prognostic indicators such as stroke type, Oxfordshire group, blood sugar and age. Extrapolation of our results suggests that the reduction in cholesterol of 25% achieved within the 4S study²⁰⁴ would result in a 13% increase in stroke mortality.

There is no established biological mechanism that explains these results but cholesterol is known to have effects on the vasculature and is essential for normal membrane fluidity. High cholesterol modifies the action of platelets such that exposure to cholesterol LDL enhances platelet aggregation by its action on platelet activating factor [PAF].²¹⁴ Rabbits fed on a high cholesterol diet therefore have larger experimentally induced infarcts associated with an increase in platelet thrombi deposition within the infarct.²¹⁵ Exposure to high levels of cholesterol also reduces the responsiveness of large but not small vessels to vasodilatory stimuli.²¹⁶ All of these effects would therefore suggest that a higher cholesterol would predispose to a poorer outcome from a stroke event.

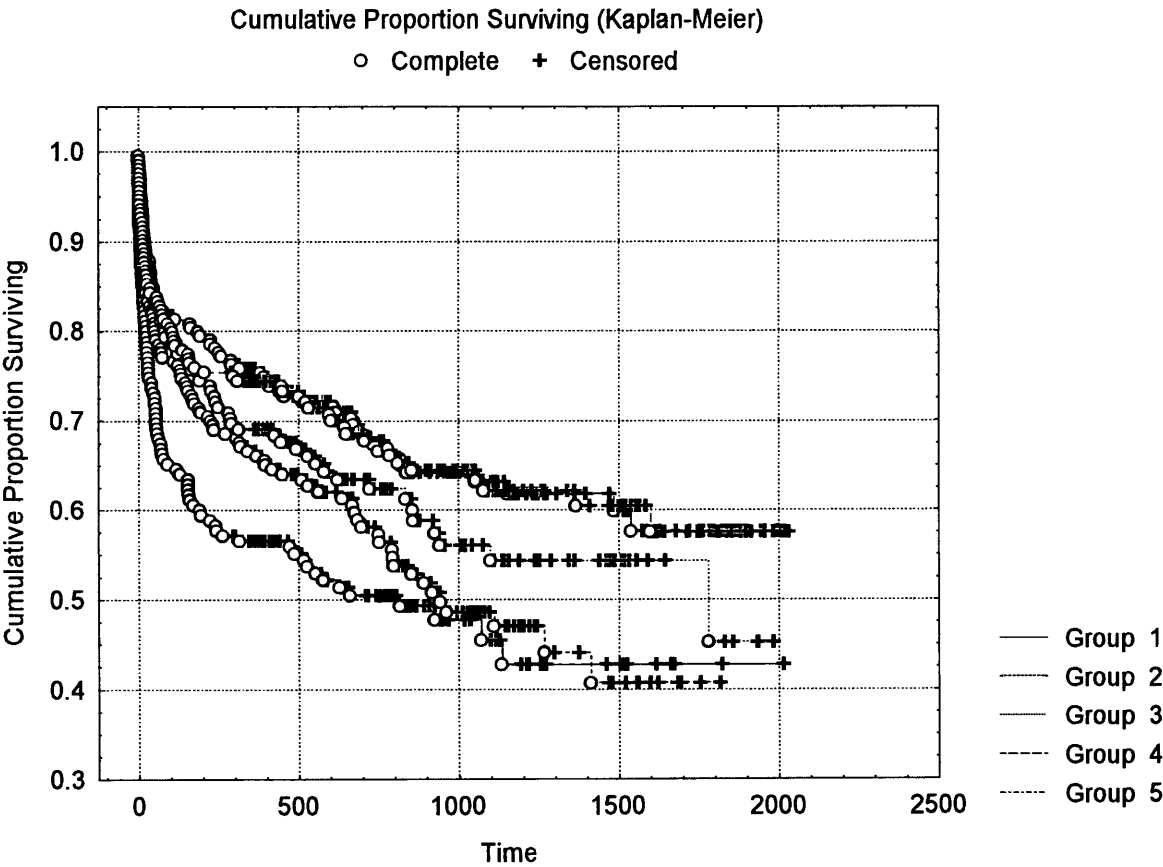
High cholesterol may exhibit a neuroprotective effect by modulating the action of the enzyme gamma-glutamyl transpeptidase (GGT) and acetylcholinesterase (AChE). GGT activity is increased while AChE activity is reduced following a high cholesterol diet.²¹⁷

GGT has a role in amino acid uptake and transport. Thus its increase in patients with higher cholesterol could reduce the neurotoxic effects of excitotoxic amino acids. Early survival does not however appear to be affected by cholesterol, rather it appears that the difference in the respective survival curves increases gradually over time. This suggests that the protective mechanism may have a more prolonged effect. Cholesterol therefore appears to be a marker for long term rather than short term survival. Why this should be so is unclear as all conventional rationale suggests that patients with higher cholesterols would have an increased risk of coincidental cardiac disease, of subsequent sudden cardiac death and of larger cerebral infarctions. Low cholesterol is however known to be associated with underlying serious illness and it might be expected that these patients would have a poorer outcome than those with higher cholesterol. Alternatively a lower cholesterol in these relatively elderly patients (average age 69.9 ± 12.4 years) may simply reflect poor nutritional status which could predispose to a poor stroke outcome.

Trials of lipid lowering agents have concentrated on middle aged patients with a low risk of stroke, and as a result it is unknown whether lipid lowering treatment is beneficial in older patients with elevated cholesterol and cerebrovascular disease. Epidemiological data suffer from failure of sub-classification of stroke types which could mask a positive relationship between infarction and cholesterol and a negative relationship with cerebral haemorrhage. Post mortem pathological data also suggest cholesterol may only increase the risk of certain sub-groups of infarcts e.g. large vessel atherothrombosis, but not lacunar events. Evidence from trials of statins suggest regression of carotid artery atheroma is possible with effective lipid lowering but whether this is clinically significant in terms of reducing cerebrovascular events is not entirely clear.

A prospective study of the incidence and outcome of stroke in relation to cholesterol in older patients (with appropriate CT and clinical diagnosis) is therefore required to define this relationship accurately. A randomised, controlled trial of lipid lowering treatment in the elderly would evaluate the safety and cost-effectiveness of lowering cholesterol in this age group and could reveal effectiveness in terms of the reduction of the incidence of both stroke and coronary events. Current practice recommends reducing cholesterol in patients > 55 years with a high risk of vascular disease despite the lack of evidence for benefit in patients > 70 years. As all previously conducted trials of lipid lowering treatment have demonstrated the benefit of lipid lowering treatment in middle aged patients these results cannot be extrapolated to the elderly population. Our data suggest lower cholesterol may have an adverse independent influence on survival following stroke but further studies to investigate and confirm this relationship are required. The efficacy of cholesterol lowering therapy as primary or secondary prevention of coronary and cerebrovascular events in the elderly remains unproven.

Figure 3.1: Kaplan-Meier cumulative survival curves for patients with stroke according to quintiles of serum cholesterol concentration: < 4.8 mmol/L (group 1, lowest curve); ≥ 4.8 and <5.6 mmol/L (group 2, second lowest curve); ≥ 5.6 and < 6.1 mmol/L (group 3, middle curve); ≥ 6.1 and < 7.0 mmol/L (group 4); and ≥ 7.0 mmol/L (group 5, top curves). Time is shown in days since stroke onset.



Chapter 4

Chapter 4

Hyperglycaemia independently predicts poor outcome following acute stroke

4.1 Introduction

Diabetic patients have worse survival and recovery prospects after acute stroke than their non-diabetic patients. In addition, hyperglycaemia in the acute phase of stroke has been established as a predictor of poor outcome in non-diabetics.

Data from the Copenhagen Stroke Study, suggested that raised blood sugar on admission predisposed to poorer functional recovery and a significantly higher mortality.²¹⁸ Smaller studies in the UK have confirmed these findings.²¹⁹ There is some dispute as to whether plasma glucose is independently associated with a poor outcome. Some investigators have noted that while admission blood glucose correlates with poor outcome, HbA1c levels and fasting or random blood sugars after the acute event have no prognostic value^{220,221}. Many investigators have gone on to suggest that hyperglycaemia itself is merely a marker of a stress response with more severe strokes eliciting a more dramatic rise in blood sugar.²²¹⁻²²⁸ This theory appears credible since poor stroke outcome correlates with increased activation of the cortisol and adrenal axis.²²⁹ No investigation into the relationship between hyperglycaemia, cortisol axis activation and poor outcome has been carried out however.

Others have suggested that hyperglycaemia influences outcome independently of stroke severity.^{218,230,231} If the latter is correct, we should investigate whether reversing hyperglycaemia in the acute phase of stroke influences its adverse effect on survival.

Rehncrona and colleagues speculated as long ago as 1981 that cerebral infarction following ischaemia is exacerbated by the build up of lactate within the brain. They demonstrated that animals exposed to global cerebral ischaemia and hyperglycaemia had larger pathological infarctions than those maintained at euglycaemic levels. They went on to postulate that the build up of acidity within the brain was an important determinant of the outcome of any vascular insult.²³² Berger and Hakim demonstrated an increased rate of cerebral oedema in hyperglycaemic stroke patients and suggested this mechanism may contribute to a poor clinical outcome.²³³

There is now good evidence from animal studies in cat and rat models of stroke that hyperglycaemia induced artificially and therefore independent of any stress response, leads to an exacerbation of the effects of cerebral ischaemia.^{234,235} In the rat MCAo model animals rendered hyperglycaemic by glucose infusion and animals infused with both insulin and glucose to maintain normoglycaemia had similar outcomes whereas those animals with blood sugar lowered to 3.4 ± 0.2 mmol (\pm SE of the mean) had 50% smaller infarcts on histological examination. This would suggest it was the correction of hyperglycaemia that had the neuroprotective effect rather than insulin itself.

The same group went on to examine the effect of insulin directly infused into the intraventricular cisterns of rats brains. They found a significant reduction in volume of cerebral infarction following transient forebrain ischaemia in animals administered low and high dose insulin, compared with placebo infused animals, with a more dramatic effect in the high dose group. The high dose regime had a systemic blood sugar lowering effect but the low dose did not. The available animal evidence therefore suggests that both hyperglycaemia itself and possibly a reduction in insulin (or even a downregulation of insulin receptor activity within the brain) could exacerbate cerebral ischaemia by an as yet undefined mechanism, and crucially that this may be amenable to therapeutic manipulation.

We studied the effect of hyperglycaemia on stroke mortality and morbidity by assessing the effect of hyperglycaemia on outcome after adjusting for known prognostic factors. We describe our findings in a cohort of unselected patients admitted to our acute stroke unit.

4.2 Patients and Methods

The acute stroke unit of the Western Infirmary, Glasgow, serves a catchment area of 220 000. All patients who present within 72h of the onset of an acute neurological deficit, with no known alternative to a vascular cause, are admitted irrespective of age or the severity of the deficit. All patients have clinical data and results of investigations prospectively recorded. Patients undergo computed tomography to establish a diagnosis of ischaemic or haemorrhagic stroke. Magnetic resonance imaging is considered as an additional diagnostic tool, particularly in patients with suspected posterior circulation events. The aim is to complete all investigations within 72h of admission. All patients have their stroke subtype categorised on the basis of clinical features according to the Oxfordshire Community Stroke Project classification.³ This clinical classification divides patients into the following four

groups: total anterior circulation syndrome, partial anterior circulation syndrome, posterior circulation syndrome or lacunar syndrome.

Biochemistry is analysed routinely in all patients on the day of admission and early the following morning. Plasma glucose is measured on both these occasions, giving one random and one fasting glucose measurement. In this study we used the random glucose measurement for each patient if it was taken; if not, we used the fasting measurement. Glucose level was recorded both as a continuous variable and a binary one (≤ 8 mmol/L, normoglycaemic; > 8 mmol/L, hyperglycaemic). The upper limit of the normal range for fasting plasma glucose level is 6.5 mmol/L. Since not all glucose measurements taken in our study were fasting, 8 mmol/L was used as the cut-point for hyperglycaemia. Other potential prognostic variables which we considered were: age, stroke type (ischaemic or haemorrhagic), admission blood pressure (systolic and diastolic), smoking status (non-smoker, ex-smoker or current smoker), resolution time of symptoms (≤ 72 h or > 72 h), and Oxfordshire community stroke project category.

The subjects in this study presented to our acute stroke unit between June 1990 and December 1993. Previously diagnosed diabetics were included in the study but the data from these patients were analysed separately since there is evidence that hyperglycaemia affects outcome differently in diabetic patients.²¹⁸

Survival and placement follow-up were by record linkage²¹⁰ to the Scottish Deaths Register and to a national database of hospital discharge records. The method of record linkage has been validated previously in an epidemiological study of hypertension,²¹¹ and has also been used for monitoring end-points and adverse events in a large clinical trial.²¹² Record linkage provides reliable patient follow-up; however, admissions to private hospitals or institutions outwith Scotland are not detected. Outcome placement was coded as: alive at home, alive

in care, or dead. This placement information was recorded at two, three, six and twelve months after admission.

Baseline variables in diabetic and non-diabetic patients were compared using χ^2 tests for discrete variables and Mann-Whitney tests for continuous variables. Differences in the distributions of potential prognostic variables between placement categories at three months were assessed by χ^2 test for discrete variables and Kruskal-Wallis analysis of variance for continuous variables. The main analysis used Cox's proportional hazards regression model²¹³ to estimate the effect of hyperglycaemia on survival after stroke. A separate baseline survival function was fitted for each of the four Oxfordshire community stroke project categories since including Oxfordshire classification as an explanatory variable was unlikely to fulfil the proportional hazards assumption. The effect of plasma glucose level was determined after entering other significant prognostic variables (selected from age, stroke type, resolution time of symptoms, smoking status, and systolic and diastolic blood pressure). The assumption of proportional hazards was checked for all variables included in the model. The effect of hyperglycaemia on outcome was further explored by coding three-month outcome as good (alive at home) or poor (alive in care, dead) and then performing a stepwise logistic regression analysis.²³⁶ We tested whether hyperglycaemia was independently associated with this outcome after adjusting as necessary for age, time to resolution of symptoms, stroke subtype, Oxfordshire classification category, smoking status, and systolic and diastolic blood pressure. In the proportional hazards and logistic regression analysis, a quadratic relationship between blood pressure and outcome was permitted.

4.3 Results

811 patients with computed tomography confirmed acute stroke and plasma glucose data were included in the study. In 624 cases (77%) of patients the plasma glucose was measured on admission; in the remaining 23% the measurement was taken early on the morning after admission. Plasma glucose was measured at a mean of 3.6 hours after admission to the stroke unit, and a mean of 14.4 hours after stroke onset. Sixty-one (8%) patients were diabetic, seven (1%) being insulin-dependent. The characteristics of these patients are compared with those of non-diabetic patients in table 4.3.1. As expected median plasma glucose level and the proportion of patients with hyperglycaemia was higher in the diabetic group. Our main analysis was restricted to the 750 non-diabetic patients. Fifteen patients were lost to follow-up for placement (due to failure of hospital discharge record linkage) but not for survival. Mean follow-up time was 1.65 years. Table 4.3.2 shows the number of patients in each outcome category over time. Table 4.3.3 gives the distributions of patient variables across the three placement categories. Table 4.3.4 shows the results of the proportional hazards modelling. Hyperglycaemia led to higher mortality, even after adjusting for other prognostic variables. Increased systolic and diastolic blood pressure were not significant linear or quadratic predictors of poor survival and were not included in the final proportional hazards model. Similarly, smoking status did not predict survival and was excluded from the model. The assumption of proportional hazards held for all variables except plasma glucose level (continuous). This variable was therefore removed from the model and plasma glucose was considered as a binary variable. Figure 4.3.1 gives the Kaplan-Meier survival curves for patients with and without hyperglycaemia, at each level of the Oxfordshire classification.

Hyperglycaemia also predicts poor outcome at three months: this variable significantly ($p=0.0003$) improved prediction of three month outcome (alive at home vs in care or dead) by logistic regression after adjusting for age, time to resolution of symptoms, stroke subtype, and Oxfordshire classification category. Systolic and diastolic blood pressure were not included in the logistic regression model since they were neither linear nor quadratic predictors of outcome. Smoking status did not predict outcome and was excluded from the logistic regression model.

4.4 Discussion

Our results show that hyperglycaemia is a predictor of higher mortality and morbidity after acute stroke, independently of other adverse prognostic factors such as older age, type and severity of stroke, and non-reversibility of neurological deficit. The effect of hyperglycaemia on mortality is large: the estimated relative hazard of 1.87 is greater than that for haemorrhagic versus ischaemic stroke, and equivalent to adding more than twenty years to a patient's age.

Our results suggest that hyperglycaemia is not solely a stress response to neurological insult, since it predicts outcome after taking other prognostic factors into account. Indeed, the relative risk conferred by hyperglycaemia is greatest in patients with lacunar stroke.

Previous studies which concluded that hyperglycaemia was a stress response, based on a correlation between stroke severity and plasma glucose level,^{226,227} did not consider whether hyperglycaemia independently predicted outcome after adjusting for stroke severity. Van Kooten et al²³⁰ demonstrated that norepinephrine levels were associated with stroke severity, but could not find significant relationships between catecholamine and plasma glucose level or between glucose level and stroke severity. They concluded that elevated plasma glucose in non-diabetic stroke patients could not be explained by a stress response. Jorgensen et

al²¹⁸ found a correlation between glucose level and stroke severity but found that glucose level independently predicted outcome after adjusting for stroke severity.

We sought to correct for admission blood pressure in our modelling of survival, since elevated blood pressure after stroke admission may be due to both mental stress of hospitalisation^{59,190} and physical stress of neurological damage. However, neither systolic nor diastolic blood pressure was associated with outcome. In addition, diastolic blood pressure was not significantly correlated with plasma glucose level (Spearman's rank correlation coefficient $r_s = 0.053$, $p = 0.0819$) while systolic blood pressure was only weakly correlated with plasma glucose level ($r_s = 0.131$, $p = 0.0003$). This further indicates that elevated plasma glucose is not due to a stress response after acute stroke.

It is likely that in many of the hyperglycaemic patients in our study, the elevated plasma glucose was of a long-standing nature. Other studies^{218,223,224,230,231} investigated this by measuring glycosylated haemoglobin HbA_{1c} and inferred that elevated HbA_{1c} levels indicated a long pre-stroke history of hyperglycaemia. HbA_{1c} is not routinely monitored in our stroke unit and we were thus unable to estimate the prevalence of previously undiagnosed diabetes.

The mechanism by which hyperglycaemia might influence stroke outcome is uncertain. Both acute and chronic hyperglycaemia are associated with increased oedema and infarct size,²³⁷ and with reduced cerebral blood flow and cerebrovascular reserve.²³⁸ Ischaemia leads to a slowing of the oxidative glucose metabolism and an increase in anaerobic glycolysis. The concentration of lactic acid increases locally as a result. Hence intracellular pH is lowered and cells die or become dysfunctional.²³² Hyperglycaemia exacerbates such changes.^{239,240} Experimental evidence suggests hyperglycaemia may increase lactate production in two ways: either directly in the severely ischaemic brain by increasing available glucose, or indirectly in the case of incomplete cerebral ischaemia by inhibiting mitochondrial respiration and glucose oxidation.²⁴⁰ Such increased lactate production in the ischaemic penumbra may

lead to poorer outcome. The above mechanisms may also cause a worse outcome in hyperglycaemic primary intracerebral haemorrhage, the excess lactate generation occurring in the area of ischaemia around the site of the haemorrhage.

Our results suggest that a randomised trial of glucose control in hyperglycaemic stroke patients is warranted. Randomisation should be soon enough after stroke onset to allow treatment during the “window of opportunity” for pharmacological intervention. Recently reported studies suggest that this time window lasts for up to three⁴ or even twelve¹⁷⁸ hours after stroke onset.

Table 4.3.1 Comparison of diabetic and non-diabetic patients

	Diabetic n=61	Non-diabetic n=750
Median age	69	70
Male sex (%)	34 (56)	371 (49)
Median plasma glucose (mmol/L) *	11.1	6.5
Hyperglycaemia (%) †	42 (69)	162 (22)
Smoker (%) †	11 (18)	326 (43)
Median diastolic blood pressure (mm Hg)	90	90
Median systolic blood pressure (mm Hg) †	170	160
Haemorrhagic stroke (%)	4 (7)	105 (14)
Symptoms resolved within 72h (%)	7 (11)	92 (12)
Oxford classification		
Total anterior circulation syndrome	12 (20)	173 (23)
Partial anterior circulation syndrome	22 (36)	259 (35)
Posterior circulation syndrome	4 (7)	78 (10)
Lacunar syndrome	21 (34)	217 (29)
Other	2 (3)	23 (3)

Figures are numbers of patients (percentage of patients) unless otherwise stated

*** Mann-Whitney test significant at p<0.0001**

† Chi-squared test significant at p<0.0001

‡ Mann-Whitney test significant at p<0.05

Table 4.3.2: Numbers of patients in each outcome category over time

	2 months	3 months	6 months	12 months
Alive at home	410 (56)	441 (60)	453 (62)	444 (60)
Alive in care	173 (24)	129 (18)	91 (12)	68 (9)
Dead	152 (21)	165 (22)	191 (26)	223 (30)

Figures are number of patients (percentage of patients at each time)

Fifteen patients were lost to follow-up for placement

Table 4.3.3 Distribution of variables with three month placement

Variable	Alive at home (n=441)	Alive in care (n=129)	Dead (n=165)
Median age [†]	68	75	72
Male sex* (%)	239 (54)	51 (40)	78 (47)
Median plasma glucose [†] (mmol/L)	6.2	6.7	7.3
Hyperglycaemia [†] (%)	68 (15)	27 (21)	66 (40)
Smoker (%)	206 (47)	50 (39)	63 (38)
Median diastolic blood pressure (mm Hg)	90	90	90
Median systolic blood pressure (mm Hg)	160	164	160
Haemorrhagic stroke [†] (%)	36 (8)	21 (16)	45 (27)
Symptoms resolved within 72h [†] (%)	82 (19)	4 (3)	5 (3)
Oxford classification[†] (% within outcome group)			
Total anterior circulation syndrome	47 (11)	44 (35)	80 (50)
Partial anterior circulation syndrome	167 (39)	38 (31)	46 (29)
Posterior circulation syndrome	60 (14)	2 (2)	15 (9)
Lacunar syndrome	154 (36)	40 (32)	19 (12)

[†] **Kruskal-Wallis analysis of variance showed significant differences between outcome groups, p<0.001**

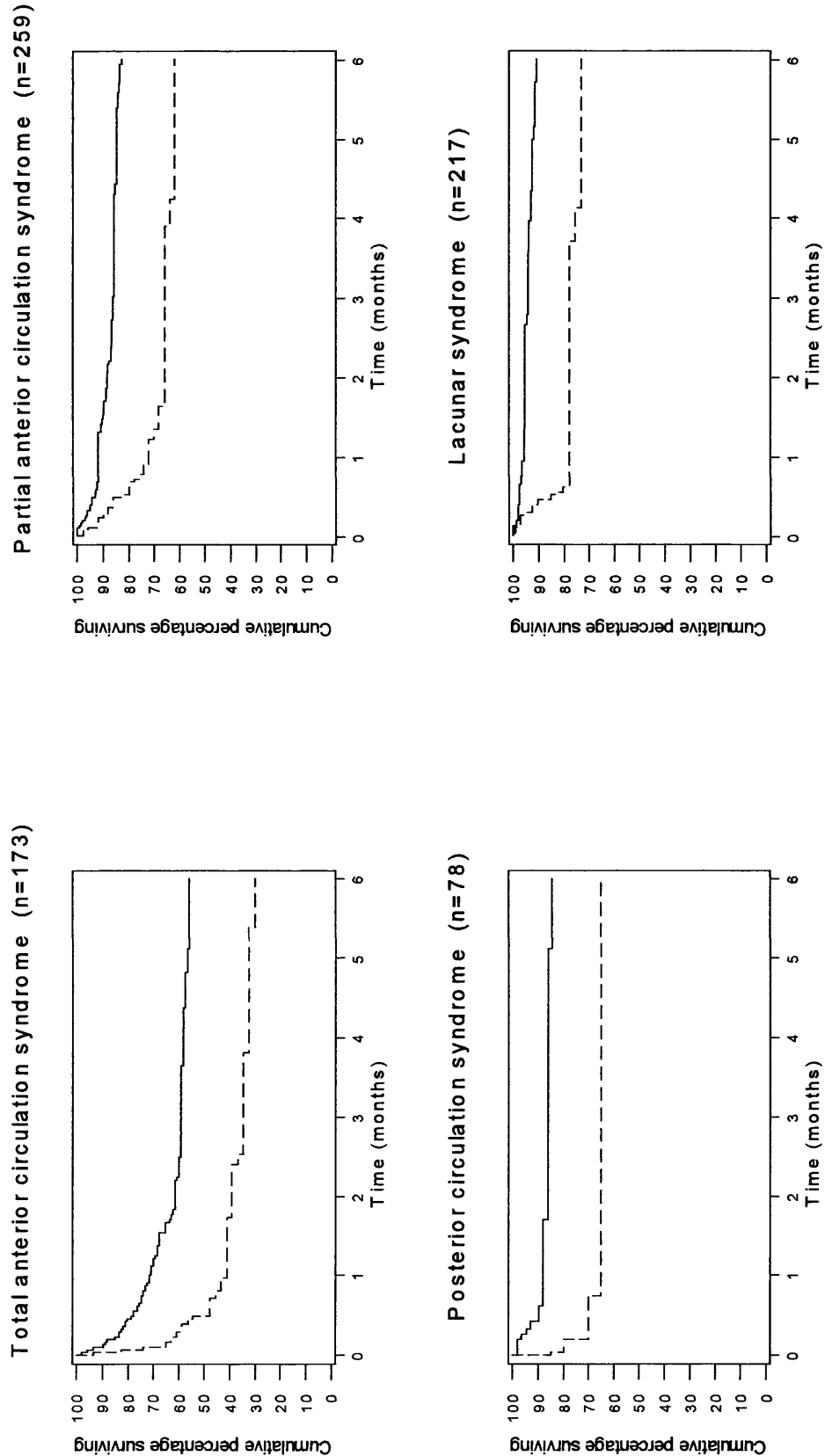
*** Chi-squared test showed significant differences between outcome groups, p<0.01**

[‡] **Chi-squared test showed significant differences between outcome groups,**
p<0.0001

Table 4.3.4: Proportional hazards modelling of mortality

Variable	Relative hazard	95% confidence interval	p-value
Hyperglycaemia	1.87	1.43 to 2.45	<0.0001
Increasing age (per decade)	1.36	1.21 to 1.53	<0.0001
Symptoms remaining after 72h	2.15	1.15 to 4.05	0.015
Haemorrhagic stroke	1.67	1.22 to 2.28	0.001

Figure 4.1: Kaplan-Meier survival curves for hyperglycaemic (-----) and normoglycaemic (——) patients, for each of the four Oxford classification categories.



Chapter 5

Chapter 5

The safety and tolerability of GV150526 (a glycine receptor antagonist) in patients with acute stroke [A double blind, randomised, placebo controlled, parallel group, ascending dose study]

5.1 Background

GV150526 is a novel antagonist at the glycine site of the NMDA receptor complex. Antagonism at this site specifically and selectively blocks activation of the NMDA receptor complex. The structure of GV150526 is shown in figure 5.1.1. GV150526 has been evaluated in animal models of stroke which have demonstrated efficacy in reducing infarct volume and improving neurological outcome.

5.2 Preclinical pharmacology

In the rat model of middle cerebral artery (MCA) occlusion GV150526 is a potent neuroprotective agent, with a putative neuroprotective concentration of 10-30 µg/mL²⁴¹ (figure 5.2.1). It is free from cardiovascular and behavioural effects at doses of up to 30mg/kg in rat or mouse and 12 mg/kg in dogs.^{242,243} Animal and human volunteer studies suggest GV150526 is less likely to cause the adverse effects seen in studies of NMDA antagonists (sedation, agitation, catatonia, nausea, GI upset, dyspepsia). Adverse reactions noted in animal toxicity testing included local injection site irritation, mild sedation, discoloured red urine, and renal tubular dilatation in rats at a dose of 200mg/kg (Glaxo laboratories, Verona, unpublished data). This was considered to be the maximal tolerated dose in rodents. Doses up to 30 mg/kg had no effect on memory or muscle tone in mice

with no evidence of neuronal vacuolation.²⁴⁴ Toxicology studies in dogs demonstrated increases in hepatic enzymes with concomitant increases in liver weight and minimal bile duct proliferation associated with mean systemic exposure of 210 µg/mL. These changes had diminished by the end of the recovery period.

Pre-clinical pharmacokinetics from animals indicate the clearance and volume of distribution of GV150526 are low suggesting limited tissue distribution. The plasma elimination half-life in the rat was estimated as 6.5 hours and in the dog 2.5 hours with the major route of elimination by biliary excretion. In-vitro plasma protein binding studies confirm high protein binding (> 99%) mainly to albumin.

5.3 Clinical experience

Prior to this study GV150526 had been given to 17 young healthy volunteers and 24 elderly volunteers, in doses ranging from 1 to 400mg (Glaxo laboratories, Verona).

Only minor adverse events were reported and there was no change in vital signs or laboratory safety data attributable to treatment. Symptoms potentially attributed to treatment included tiredness, headache, neck-ache, flatulence, loose bowels and sore throat.

Pharmacokinetic data from these studies (dose range 1-400mg) suggest clearance and volume of distribution are low in humans with an elimination half life of 19 hrs. C_{max} was linearly related to dose throughout the 1-400 mg range.

5.4 Aims and objectives

Part A

Primary

- To assess safety and tolerability of ascending doses of GV150526 in patients with acute stroke (doses 50, 100, 200, 400, 800mgs).

- To assess clinical pharmacokinetics of GV150526 in patients with acute stroke.

Secondary

- The power of the study was not sufficient to assess the therapeutic efficacy of GV150526 but preliminary data on stroke outcome were gathered.

Part B

Primary

The objectives of the second study were to assess the safety and tolerability of a bolus dose selected from the initial study and followed by repeated infusions selected to maintain putatively neuroprotective concentrations during 60 hours.

Secondary

Preliminary outcome data were collected but the study was not powered to demonstrate efficacy.

5.5 Patients and methods

5.5.1 Study design

Part A

This was a double blind randomised (ratio of 3:1 active: placebo) placebo controlled, parallel-group, ascending single intravenous dose study in acute stroke patients. Eight patients were to be enrolled into each dosage group.

PartB

This was a double blind, randomised, placebo controlled, parallel-group, ascending multiple intravenous dose study. Each patient received a loading dose of 800mg GV150526 followed by 5 repeat infusions at 12 hourly intervals.

Ethics and consent

The study design and consent form were approved by the West Ethics Committee of the Western Infirmary, Glasgow. Written informed consent was obtained from the patient or relatives before subjects were randomised to receive either active drug or placebo. Where written consent could not be obtained, consent was accepted verbally, but only in the presence of an independent witness unconnected to the trial. On rare occasions when patients were unable to give written or verbal consent, consent was accepted from the next of kin in the presence of an independent witness.

5.5.2 Patient selection

Inclusion criteria

- Males aged over 18 years, and post menopausal or surgically sterilised females.
- Patients suffering acute ischaemic stroke within 12 hours of study entry. For patients with night time stroke, time of waking was taken as time of onset.

Exclusion criteria

Patients with the following were excluded from the study

- Coma (unable to localise painful stimulus)
- Known serious hepatic or renal abnormalities.
- Receiving investigational drug within previous 3 months.

- Acute unstable systemic illness other than acute stroke.
- Known underlying seizure disorder.
- Anti-coagulation with warfarin.
- Patients taking > 1gram aspirin in the previous 24 hours.

5.5.3 Dosing regimen

Part A

Within each study panel, 8 patients were allocated placebo or active treatment according to the randomisation code. The dose regimens are summarised below.

Part A: Bolus

Group	Dose mg	Infusion time mins	Volume infused mls
1	50	7.5 min	62
2	100	15 min	125
3	200	30 min	250
4	400	60 min	500
5	800	240 min	1000

N.B Group 5: 400mL in 500ml over 60 mins + 400mg in 500mL over 180 mins.

Part B

Within each dosage group 8 subjects (6 active and 2 placebo) received treatment as allocated in the table below. All patients receiving active treatment were administered a loading dose of 800mg over 4 hours. The first maintenance infusion commenced 12 hours after the start of the loading dose. The maintenance dose was administered at a rate of 500 mL/ h. The duration of each infusion was therefore variable and is shown in the table below.

Part B: Bolus + 60 hour infusion

group	Loading dose	Maintenance infusion	duration of each maintenance infusion
1	800mg / 4 hours	100mg bd × 5 infusions	15 minutes
2	800mg / 4 hours	200mg bd × 5 infusions	30 minutes
3	800mg / 4 hours	400mg bd × 5 infusions	60 minutes

5.6 Study procedures

5.6.1 Screening

Following admission to the acute stroke unit patients underwent initial clinical and neurological assessment by a member of the medical staff. Only patients who met the inclusion criteria and for whom consent was obtained were entered into the study.

5.6.2 Safety

The following analyses were carried out

Haematology	Biochemistry	Urinalysis
haemoglobin	sodium	glucose
white cell count	chloride	protein
red cell count	potassium	blood
platelet count	bicarbonate	
haematocrit	urea	
	creatinine	
coagulation	calcium	
prothrombin time	phosphate	
activated partial thromboplastin time	albumin	
thromboplastin time	protein	
fibrinogen	bilirubin	
	alkaline phosphatase	
	AST	
	ALT	
	GGT	

Blood samples were taken for biochemical screening, urinalysis performed and 12 lead ECG recorded pre-dosing and at 4, 60 and 96 hours. Continuous ECG monitoring was performed throughout all infusions. CT or MRI scanning was carried within 3 days on all patients to

confirm the diagnosis of stroke. Scanning was not a prerequisite to study entry but patients with CT findings inconsistent with a diagnosis of stroke had study drug discontinued and remained within the study for collection of safety data.

Coagulation was assessed using prothrombin time at baseline, 24 and finally 72 hours.

Biochemistry was analysed by the routine laboratory analyser at the Biochemistry Department, Gartnavel General Hospital, Glasgow. Haematology was performed by automatic counter at the Western Infirmary Haematology Department. Urinalysis was carried out using Multistix SG (Bayer Diagnostics, Basingstoke, Hampshire UK) and the presence of blood, protein and glucose noted.

5.6.3 Tolerability

Adverse event enquiries

Adverse event reports were collected at the start of the infusion and at the following intervals: 2h, 4h, 8h, 12h, 24h, 36h, 48h, 72h, 1 week, 1 month. Brief general physical examinations were carried out at baseline, 24 h, 3 days, 1 week and 1 month wherever possible. A functional assessment (Barthel index) was recorded at 1 month.

5.6.4 Vital signs

Blood pressure and pulse recordings were made using Marquette semi-automatic oscillometric monitoring equipment (Marquette Electronics Inc. Wisconsin USA) and repeated at frequent intervals during drug dosing.

5.6.5 Pharmacokinetics

Blood samples (2mL) were taken from a cannula sited in the ante-cubital fossa of the non-infusion arm, at regular and frequent intervals for assay of the main metabolite of GV150526

(GV150526X). The sampling times varied according to the dose administered. All time points were nominal and precise times of sampling were documented in the case report forms.

Samples were stored on ice immediately after collection and centrifuged (3000rpm for 15 mins at 4°C). Plasma was then transferred to a plastic vial and frozen ($\leq -18^{\circ}\text{C}$) within 3 hours of collection.

Urine was continuously collected where possible over the 0-24 and 24-48 hr period and assayed for GV150526X and creatinine. At the end of the collection period urine was stored frozen ($\leq -18^{\circ}\text{C}$) in a plastic container. The pharmacokinetic analysis was performed in the Drug Metabolism Department Glaxo S.p.A., Verona.

Plasma and urine were transported from the Acute Stroke Unit on dry ice. Samples were stored at $\leq -18^{\circ}\text{C}$ pending analysis. Plasma concentrations of GV150526X, the main metabolite of GV150526 were determined using an online solid phase extraction, with fully automated cartridge exchange with HPLC separation and UV detection. The validated limit of quantification for this analysis was $0.2\mu\text{g/mL}$.

The binding of GV150526X to plasma proteins was determined in the C_{max} samples from all patients. Protein binding was determined by equilibrium dialysis and assay of free fractions by HPLC.

GV150526X in urine was assayed using a HPLC separation with direct injection of diluted urine and UV detection. The validated limit of quantification for this method in the calibration range was $0.025\mu\text{g/mL}$.

Part A

Standard non-compartmental methods were applied for the pharmacokinetic analysis. The maximum concentration (C_{max}), the time taken to reach maximum plasma concentration (t_{max}), the area under the plasma concentration time curve from zero to the time of the last measurable plasma concentration (AUC_{last}), the area under the curve from zero to infinity (AUC_{∞}), the terminal rate constant (λ_z), the associated terminal half-life ($t_{1/2}$) and the mean residence time corresponding to an intra-venous bolus dose (MRT_{iv}) were calculated using PCNONLIN (release 4.2a, non-compartmental model 202).

AUC_{last} was calculated by the linear trapezoidal method, λ_z was estimated by log-linear regression of those data points that describe the terminal log-linear decline in the plasma concentration / time profile, and $t_{1/2}$ was calculated as $\ln 2 / \lambda_z$. AUC_{∞} was calculated as $AUC_{last} + C_{last} / \lambda_z$, where C_{last} was the last measurable concentration; the mean residence (MRT_{iv}) was calculated as $(AUMC_{\infty} / AUC_{\infty}) - T/2$ where $AUMC_{\infty}$ is the area under the first moment of the curve and T was the infusion time.

The percentage of AUC_{∞} obtained by extrapolation ($\%AUC_{ex}$) was calculated as

$C_{last} * 100 / (\lambda_z * AUC_{\infty})$. Total plasma clearance (CL) was calculated as $Dose / AUC_{\infty}$, apparent volume of distribution (V_d) as CL / λ_z , volume of distribution at steady state (V_{dss}) as $CL * MRT_{iv}$. GV150526X excreted in the urine were estimated from the concentrations measured in urine samples and the collected urine volume. Renal clearance (CL_r) was calculated as A_e / AUC_{∞} where A_e is the total amount of GV150526X excreted in the urine.

Protein binding was assessed, from samples taken at the end of the infusion, using equilibrium dialysis HPLC.

The relationship between pharmacokinetic parameters and patient age and weight was explored using linear regression analysis. Regression analysis plots were generated together with the predicted regression line. The significance of the correlation was determined from the slope of the regression.

A compartmental analysis was also performed using a two compartment model with constant intravenous input and first order output. The volume of the central compartment V_1 , the elimination rate constants (k_{12}, k_{21}) were estimated by nonlinear reweighted least squares regression analysis (PC NONLIN 4.2 model 9, reweight -2).

Part B

In part B the appropriateness of a two or three compartment model with intermittent intravenous infusions and first-order output was assessed. Actual infusion and blood sampling times were used in the analysis.

The volume of central compartment (V_c), the elimination rate constant from the central compartment (k_{10}) and the distribution rate constants (k_{12} , k_{21} , k_{13} , k_{31}) were estimated by non-linear least squares regression analysis (WinNonLin 1.1, Models 9 and 13).

Concentration data were weighted using either $1/Y$, $1/Y^2$ or no weighting as appropriate, where Y was the model predicted concentration value. Assessment of goodness of fit was based on observed/predicted concentration-time profiles, residual plots and Akaike Information Criterion(AIC). The results indicated that a two compartment model best described the concentration/time profile of GV150526X.

The maximal plasma concentration (C_{max}) was determined directly from the observed concentration data. Clearance (Cl), steady state volume of distribution (V_{dss}) and terminal half life ($t_{1/2}$) were determined as secondary parameters by WinNonLin. Intercompartmental clearance (Cl_{12}) was estimated from the product of V_c and k_{12} . The average steady state concentration (C_{ss}) for each patient was determined from the maintenance dose infusion rate and clearance.

Microsoft ® Excel v 5.0 was used for all calculations and descriptive statistics.

5.6.6 Outcome

NIH stroke scale (appendix 1) assessments were made at baseline, 24 hours, 72 hours, 1 week and 1 month. Barthel index (appendix III) functional assessment was undertaken at the completion of the study i.e. 1 month.

5.7 Results

5.7.1 Recruitment and demographics

A total of 66 patients were recruited to parts A and B of the study. 18 patients in total received placebo (12 within part A and 6 within Part B) and 6 each received bolus doses of 50, 100, 400 and 800mg GV150526. Due to a dosing error only 5 patients received the 200mg dose, (the sixth received 400mg over 30 minutes). In part B: 6 patients were administered 100 and 200mg bd and 7 patients received the highest dose of 400mg bd. The demographic details of patients entering parts A and B of the study are summarised in the tables below.

Demographics: Part A

	placebo	50mg	100mg	200mg	400mg	800mg
number of patients	12	6	6	5	6	6
sex						
male	7	2	4	2	5	2
female	5	4	2	3	1	4
age (yrs)						
mean	73.4	66.8	72.4	62.2	68.1	72.8
SD	13	22.1	17.8	5.7	9.9	11.3
weight(kg)						
mean	68.3	72.5	76.4	62.2	76.7	65.8
SD	7.3	8.2	23	13.5	17.8	18.7
height(cm)						
mean	166.3	163.5	166	165	165.3	166.6
SD	7.2	10.2	14.8	8.5	6.6	9.1

Demographics: Part B

	placebo	100mg bd	200mg bd	400mg bd
number of patients	6	6	6	7
sex				
male	3	4	2	2
female	3	2	4	5
age (yrs)				
mean	78	67	69	71
SD	8.6	10.3	11.6	14.2
weight (kg)				
mean	69.5	64.5	63.2	61.4
SD	8.1	9.8	5.5	8.5
height (cm)				
mean	169	166	163	162
SD	8.1	9.8	5.5	8.5

5.7.2 Compliance

Protocol violations

Six patients were dosed after 12 hours of onset. In each case the patients had been assessed and consent obtained before 12 hours but the start of the infusion was delayed due to practical difficulties in obtaining venous access and setting up the infusions. These protocol violations are described in the tables below.

Part A

patient	dose	violation
6	placebo	time to treatment : 12 hours 55 minutes
23	placebo	time to treatment: 12 hours 20 minutes
35	placebo	time to treatment: 13 hours 30 minutes
3	50mg	time to treatment: 12 hrs 15 minutes

Part B

patient	dose	violation
96	200mg bd	time to treatment: 15hrs 30 mins
103	400mg bd	time to treatment: 12hrs 40 mins

5.7.3 Serious adverse events

Mortality

There were 8 deaths out of a total of 66 patients: 7 in part A and one in part B. The fatal events are summarised in the table below.

patient number	treatment	fatal event	time after infusion commenced
35	placebo	further stroke	14 days
51	placebo	further stroke / bronchopneumonia	21 days
17	100mg	bronchopneumonia	29 days
24	100mg	myocardial infarction	20 days
65	800mg	stroke + complicating infection	17 days
66	800mg	stroke	49 days
71	800mg	GI haemorrhage	< 1 hour
Part B			
91	200mg bd	extension of stroke	11 hours

Deaths: Part A

Placebo

2/12 (17%) placebo patients died at 14 and 21 days after stroke as a result of extension of stroke and a combination of recurrent stroke + bronchopneumonia respectively: subjects 35 and 51.

800mg bolus

Three patients in the 800mg group died as a result of

1. extension of stroke on day 49 (subject 66);
2. complicating infection on day 17 (subject 65);
3. gastrointestinal haemorrhage and myocardial infarction which presented less than 1 hour after the start of the infusion (subject 71).

A post mortem in the last patient revealed gastric erosions and a leaking aortic aneurysm.

The plasma urea on admission was subsequently found to be elevated consistent with a recent prior GI haemorrhage.

Deaths in part B

Patient number 91 (200mg bd dose group) died 11 days after the start of treatment. The patients deterioration was thought to be due to the CT confirmed large middle cerebral artery infarction.

Withdrawals

Part A

Five patients were withdrawn from the study prior to their one month follow up assessments.

Two were withdrawn prior to completion of drug infusion (subjects 17 and 71).

During infusion

100mg

- Subject 17 developed unstable atrial fibrillation immediately prior to the infusion being started and was subsequently replaced with subject 25.
- Patient 21's conscious level deteriorated 23 hours after infusion commenced and later recovered but this was consistent with the underlying diagnosis of a moderate sized cortical intracerebral haematoma.

Post infusion

50mg

- Patient 5 in the 50mg dose group developed prolonged sinus pauses, requiring permanent pacemaker insertion 16 hours after the start of the drug infusion. A right bundle branch block pattern had been present on the ECG prior to the start of the infusion.

100mg

Patients 17 and 24 (both 100mg) developed acute left ventricular failure, 27 and 15 days after treatment respectively.

Part B

Six patients were withdrawn prior to their 1 month assessments. Four withdrawals were prior to completion of treatment.

During infusion

Placebo

Patient 99 was withdrawn due to extension of stroke.

100mg bd

Patient 98 was withdrawn following the loading dose after the onset of vomiting and positive faecal occult blood.

400mg bd

- Patient 97 exhibited fluctuating levels of consciousness, neck stiffness, drowsiness, and confusion after 2 days infusion treatment and was subsequently withdrawn from the study.
- Patient 102 developed an infusion site cellulitis 24 after commencing treatment and was withdrawn prior to completion of the infusion regimen.

Post infusion

200mg bd

- Patient 91 was withdrawn 1 week after treatment due to deteriorating conscious level which led to death on day 11 of the study.
- Patient 93 was discharged from hospital and subsequently failed to attend follow up visits.

Other serious adverse events

Part A

There was one additional serious adverse event in part A of the study. Patient 72 suffered a recurrent transient ischaemic attack 3 days after enrolment.

Part B

All serious adverse events reported in part B of the study were either fatal or resulted in withdrawal from the study.

CNS effects

In contrast to other NMDA receptor antagonists e.g. remacemide (chapter 6) and particularly aptiganel (chapter 7), GV150526 was exceptionally well tolerated by patients even at high doses. There was no excess of CNS adverse events reported in the actively treated patients in either study.

Other adverse events

Headache was more commonly reported in the actively treated group of part A; 5/29 vs 1/12 placebo. There were no other consistently reported side effects and no increase in reported adverse events in the actively treated groups.

In part B there was no excess of headache or other any other reported symptoms within the actively treated patients.

No serious adverse events reported in either part A or B were thought likely to be the result of drug administration. The overall death rate of 12% over a 1 month follow up period is consistent with the prognosis expected in patients with stroke. Acute stroke unit mortality for 1996 was 12.2%

Vital signs

No changes in blood pressure or heart rate consistent with drug effect were reported. All deviations in ECG findings within 1 week of drug administration are reported in the table below.

Patient no.	dose	time after start infusion (hrs)	abnormality
35	placebo	24	new anterior Q wave
54	placebo	72	bradycardia : rate 52
56	400 mg	1	ventricular bigeminy
5	50 mg	1	sinus arrest requiring pacemaker insertion
71	800 mg	1	myocardial infarction following massive GI bleed
22	100 mg	1- 72	generalised myocardial ischaemia
89	100 mg bd	60	myocardial ischaemia. ST elevation
98	100 mg bd	96	ventricular trigeminy
94	200 mg bd	60	sinus tachycardia

An analysis of changes in ventricular rate, PR, QRS, and QT intervals at the time of maximal drug concentration revealed no difference before and during infusion in treated patients from part A. The results are shown in the table below.

		V. Rate	PR	QRS	QT interval
before infusion	mean	75	185	113	392
	SD	23	18	18	20
during infusion	mean	72	182	96	395
	SD	19	20	24	18

5.7.4 Laboratory results

Part A

There were no changes attributable to study drug on laboratory screening. Bicarbonate was mildly reduced in 4/6 patients in the 800 mg, 1/6 of the 400 mg 1/6 100mg 1/6 50 mg and 2/12 placebo groups. This is likely to reflect the clinical condition of the patients within the higher (800 mg) dose group rather than any dose dependent drug effect. Minor disturbances in electrolytes were equally frequently reported in both the active and placebo groups consistent with the administration of intravenous fluids or dehydration. Increases in liver enzymes (GGT and ALT) were reported in 6/29 (21%) actively treated patients compared with 3/12 (25%) controls. In each actively treated subject the rise was transient and not severe. Mild anaemia was reported in a total of 4/29 (14%) actively treated patients compared with 1/12 (8.3%) placebo patients, but there was no increased frequency at the higher dose levels and in each case hematological indices were consistent with pre-existing iron deficiency. One patient in the 800 mg group developed a transient reduction in platelet count 72 hours after commencing drug infusion (142 at baseline → 81, recovering to 164 at 1

week). This was not associated with any abnormalities in total red or white cell count that may have suggested marrow failure.

Part B

Elevations in bilirubin, GGT and liver transaminases consistent with cholestasis were reported following repeated infusions. The effect was dose dependent and particularly prevalent at the highest dose (400 mg bd). Mean rises in liver function tests for each dose group are summarised in figures 5.7.4.1, 5.7.4.2 and 5.7.4.3. There were no changes in liver function in any placebo receiving patients.

One out of six patients receiving 100 mg had a 3 fold rise in GGT and ALT at 24 hours. This had resolved by 10 days. In the 200 mg group 2/6 patients had liver function abnormalities: one patient had an isolated 3 fold increase in ALT at 84 hours; a second patient had a 2 fold increase in bilirubin, AST and ALT at 84 hours, resolving by 10 days.

In the group receiving 400 mg bd 4/7 patients had significant rises in bilirubin and 3/7 had increases in GGT and alkaline phosphatase, consistent with a hepatic cholestatic effect. Changes in liver function tests in individual patients in the 400mg bd dose group are summarised in figures 5.7.4.4, 5.7.4.5 and 5.7.4.6.

5.7.6 Pharmacokinetics

Part A

Detectable plasma levels of GV150526X were observed in all actively treated patients at all sample times post-infusion. GV150526X had a low clearance (0.27-0.39L/hr) and a terminal half life of approximately 25-35 hours. The central compartment volume of distribution was 3-4 L, which is equivalent to plasma volume (~3L). The steady state volume of distribution was 7-10 L and is equivalent to the apparent volume of distribution for plasma proteins (~7L). The low distribution may result from high plasma protein binding (>99.9%) of GV150526X.

Pharmacokinetics appeared linear up to a dose of 100 mg. The C_{max} for the 800 mg dose was less than the predicted value from the 400 mg dose. This may be due to saturation of protein binding at higher doses leading to increased penetration of peripheral tissues. The $t_{1/2}$ was long varying between 34.9 hours in the 50 mg group and 23.0 hours in the 800 mg group. Urinary excretion was between 0.14% and 0.27% total dose and the volume of distribution was low (between 7.43 L in the 50 mg and 9.93 L in the 800 mg group) consistent with high protein binding noted in animal studies. Plasma concentrations predicted to be neuroprotective in humans from the rat MCA occlusion model were successfully exceeded, sustained and well tolerated.

Systemic clearance was consistent with results previously observed in healthy volunteers (0.3-0.42 L/hr). Renal elimination accounted for less than 1% of the total dose. The terminal $t_{1/2}$ was similar to that observed in previous volunteer studies i.e. 25-35 hrs). GV150526 has a low volume of distribution at steady state i.e. 7-13 L due to high protein binding (99.99%). Analysis of the AUC_{∞} and CL for each dose group suggests deviation from linear kinetics at doses of 400 mg and 800 mg. These observations may be the result of saturable protein binding.

Regression of C_{\max} against dose following bolus injection is shown in figure 5.7.6.1.

A summary of median pharmacokinetic parameters at each dose level is summarised below

Parameter	50mg/7.5min	100 g/15min	200mg/30min	400 mg/1 hr	800 mg/4 hr
	n=4	n=4	n=4	n=6	n=5
Cmax (µg/mL)	16.5	28.9	69.2	101	106
	(11.4-18.8)	(22.6-35)	(49.5-76.1)	(95.3-126)	(105-121)
AUC∞ (µg/mL)	167	374	646	1096	2092
	(138-211)	(267-444)	(503-998)	(954-1272)	(1399-3372)
CL (L/h)	0.3	0.27	0.32	0.37	0.38
	(0.24-0.36)	(0.23-0.37)	(0.2-0.4)	(0.31-0.42)	(0.24-0.57)
Vdss (L)	7.43	8.15	6.69	8.64	9.93
	(6.28-9.27)	(6.3-9.71)	(5.37-7.91)	(7.56-14.2)	(9.14-16.96)
t 1/2 (h)	34.9	30.8	25.2	27.4	23.0
	(25.4-46.2)	(27.3-40.5)	(21.3-26.6)	(18.5-43.7)	(19.6-52.2)
Urin. excr.(% dose)	0.24	0.25	0.27	0.14	0.21
	(0.18-0.91)	(0.22-0.61)	(0.02-0.48)	(0.05-0.55)	(0.11-0.82)
CLr(L/hr)	0.0006	0.0008	0.0008	0.0005	0.0011
	(0.0006-0.0033)	(0.0006-0.0018)	(0.0001-0.001)	(0.0002-0.0022)	(0.0004-0.0025)
% unbound at Cmax	ND	ND	0.0015	0.0034	0.0045
			(0.0011-0.0023)	(0.0017-0.0080)	(0.0028-0.0070)

Part B

GV150526 again was found to have a low clearance (0.31-0.40 L/hour) and low central (3.3-3.9L) and steady state volume of distribution (9.8-17L). The terminal $t_{1/2}$ was 29-56 hours. The low distribution results from high plasma protein binding (>99.9%). The clearance was 30% lower in females. Volume of distribution was not related to patient demographics. The free fraction of drug increased in some patients during the 60 hour treatment period, particularly in those patients with elevated bilirubin. GV150526 and its conjugated metabolites altered the elimination process of bilirubin. A significant inverse relationship was noted between patient age and clearance ($p=0.02$) and a significant direct relationship was noted between patient weight and clearance ($p=0.02$). Patient weight was highly correlated with patient age ($p<0.001$), thus the effects of age and weight on clearance could not be distinguished. No relationships were observed between volume of distribution and patient age or weight.

Concentrations achieved after administration of the three maintenance doses are shown in figures 5.7.6.2, 5.7.6.3 and 5.7.6.4.

A summary of the statistical analysis of the compartmental pharmacokinetics in part B is shown in the table below.

Summary of geometric mean (95%CI) compartmental pharmacokinetic parameters for GV150526X.

parameter	group1	group 2	group 3
dose	800mg/100mg bd	800mg/200mg bd	800mg/400mg bd
	n=6	n=6	n=7
CL (L/hr)	0.4	0.31	0.33
	(0.31-0.50)	(0.25-0.37)	(0.20-0.54)
CL ₁₂ (L/h)	0.53	0.73	0.48
	(0.36-0.79)	(0.59-0.91)	(0.20-1.25)
V _c (L)	3.36	3.33	3.9
	(2.71-4.17)	(2.54-4.35)	(3.35-4.54)
V _{dss} (L)	11.5	9.84	17.0
	(9.35-14.0)	(7.94-12.2)	(15.1-21.3)
t _{1/2α} (h)	2.14	1.63	2.73
	(1.50-3.04)	(1.22-2.18)	(1.23-5.63)
t _{1/2β} (h)	28.9	26.7	55.9
	(23.6-35.4)	(18.4-38.9)	(44.7-79.1)
Predicted C _{ss} (μg/mL)	118	118	135
	(101-137)	(107-131)	(118-144)
Urin. excr. (%dose)	0.35	0.19	0.29

5.7.7 Clinical outcome

The power of the study was not sufficient to show any trend towards efficacy. The results of the NIH Stroke scale and Barthel index follow ups are documented in figures 5.7.7.1, 5.7.7.2, 5.7.7.3 and 5.7.7.4 and not unexpectedly failed to demonstrate differences between drug and placebo treated patients. The overall outcome in the highest dose group was poorer due to the baseline severity and complications of stroke suffered by those particular individuals.

5.8 Discussion

GV150526 had no effect on blood pressure, heart rate or the electrocardiogram. No serious adverse events related to drug therapy were reported and there was no trend to altered functional outcome.

Plasma concentrations predicted to be neuroprotective in humans from the rat post MCA occlusion model were successfully exceeded and were well tolerated. Infusions of 200mg and 400mg bd sustained plasma levels of GV150526 above the concentration demonstrated to be neuroprotective in animal models of 10-39 µg/mL (figures 5.7.6.3 and 5.7.6.4).

Interestingly there were no significant adverse psychotomimetic or haemodynamic effects as frequently reported with other NMDA antagonists. While it has been suggested that the favourable side effect profile may be due to a lack of sufficient drug within the brain tissue, preclinical animal work with GV150526 did suggest that this compound would be better tolerated than conventional NMDA receptor antagonists. The lack of haemodynamic and CNS effects is consistent with the preclinical animal experimental findings.

It may be that antagonism of the glycine binding site of the NMDA receptor complex rather than the ion channel leads to neuroprotection without haemodynamic and psychotomimetic effects. This would certainly give such a compound a significant advantage over other potential neuroprotectives which are less well tolerated. Therapeutic levels of drug could reliably be obtained and sustained and patient compliance would be enhanced. Significant numbers of patients are likely to 'drop-out' of any treatment which has a psychotomimetic side effect profile even if well motivated to receive treatment.

Haemodynamic effects are of particular concern to physicians attending stroke patients. Complications of haemorrhagic transformation and brain oedema (in those patients with hypertensive responses) and exacerbation of the infarction in those with blood pressure falls, either as a direct consequence of neuroprotective treatment or as a result of treatment given to reverse a hypertensive drug effect, will complicate the prescribing of any such drug. A clean haemodynamic side effect profile is a major benefit to any compound being evaluated as a potential neuroprotective. GV150526 was free from haemodynamic effects.

Pharmacokinetic analysis suggests that GV150526 has a relatively long $t_{1/2}$ which would make intermittent infusions feasible, i.e. several 1 hour infusions over several days. This is an advantage as it does not necessitate constant intravenous infusion of drug to maintain therapeutic levels, which would potentially restrict early rehabilitation.

Constant infusions require regular supervision by nursing or medical staff and compliance is likely to be compromised by interruptions to the infusion regime which may rapidly cause a fall to sub-neuroprotective levels, particularly if the $t_{1/2}$ is short.

The abnormalities in liver function reported in the prolonged administration phase of the study raise potential concerns regarding the overall safety profile.

Patients with elevated bilirubin had increased free fraction of GV150526X, suggesting protein binding displacement. GV150526X may alter the glucuronide conjugation and / or biliary elimination of bilirubin as suggested from elevated levels of bilirubin and altered pharmacokinetics of GV150526X and its conjugated metabolite. These pharmacokinetic explanations for changes in bilirubin concentration cannot however fully explain accompanying rises in hepatic enzymes (AST and ALT) or moreover the rise in alkaline phosphatase.

It is likely that the changes observed in this study are analogous to the rise in hepatic enzymes associated with increases in liver weight and bile duct proliferation observed in studies in dogs. Reassuringly in these studies hepatic pathological changes were reversible and had largely resolved by the end of the 10 day recovery period.

While rises in liver function tests of 2-3 times the normal reference range in themselves may be of little clinical significance, particularly as all patients were asymptomatic and values returned to normal within 10 days, the cause and possible implications of these observations necessitate careful monitoring of liver function in future studies. Many useful and safe drugs exhibit similar properties, notably anticonvulsants. Asymptomatic rises in liver function are common and routine monitoring of liver function is neither required or advised. Even if future studies with GV150526 suggest mild hepatic toxicity this would not automatically preclude the clinical use of such a drug. The untreated prognosis of stroke patients is so poor that effective treatment would be acceptable to both patients and clinicians even if the drug was mildly hepatotoxic.

In summary, GV150526 appears to be a well tolerated glycine antagonist with a favourable pharmacokinetic profile to permit twice daily infusion. Minor changes in hepatic biochemistry require further monitoring. The optimal dose for phase 3 studies appears to be 800 mg as a loading infusion, followed by 200 mg maintenance infusions twice daily. This dose will rapidly exceed and maintain concentrations in excess of 10 ng/ml, whilst minimising transient liver function abnormalities.

Figure 5.1.1: Structure of GV150526

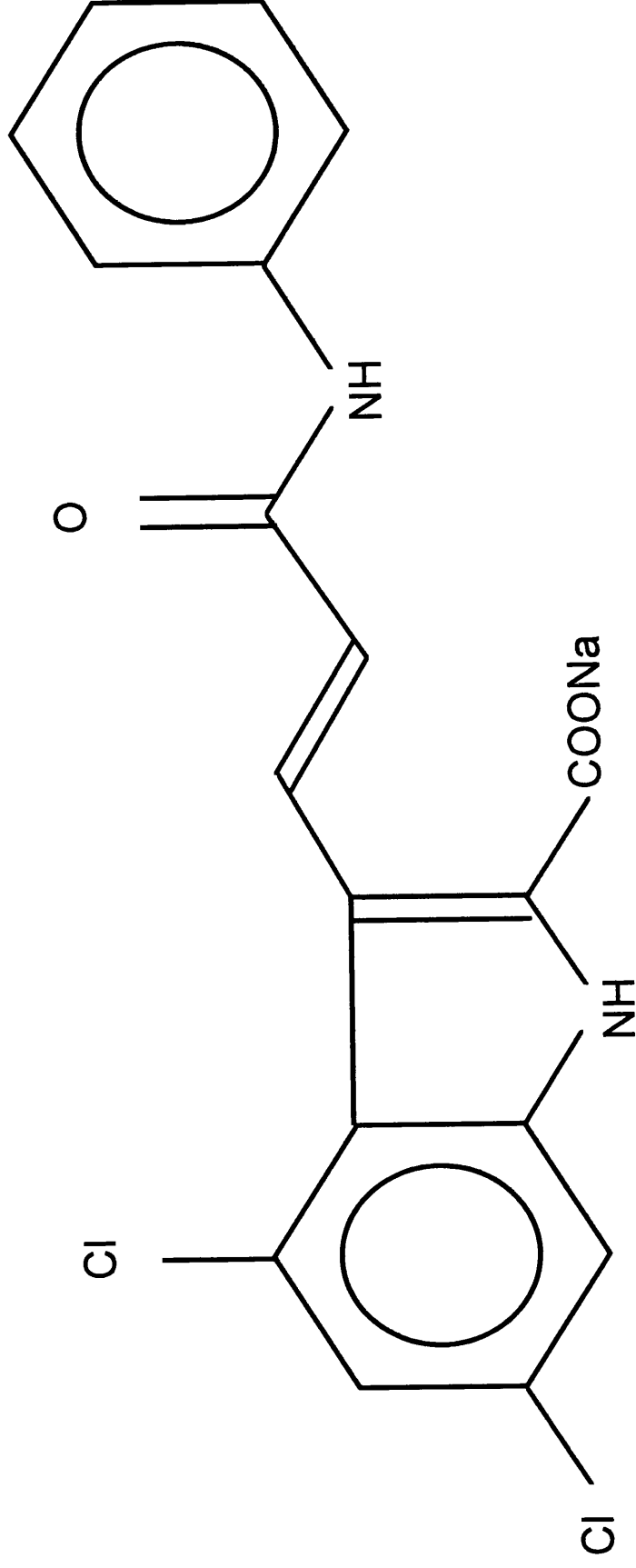


Figure 5.2.1: Time course of MCAo induced neurodegeneration (\pm sem) in spontaneously hypertensive rat and after permanent middle cerebral artery occlusion: effects of administration of GV150526 (3mg/kg,iv) at 3 hours post ischaemia. Brains evaluated histologically at time points indicated.

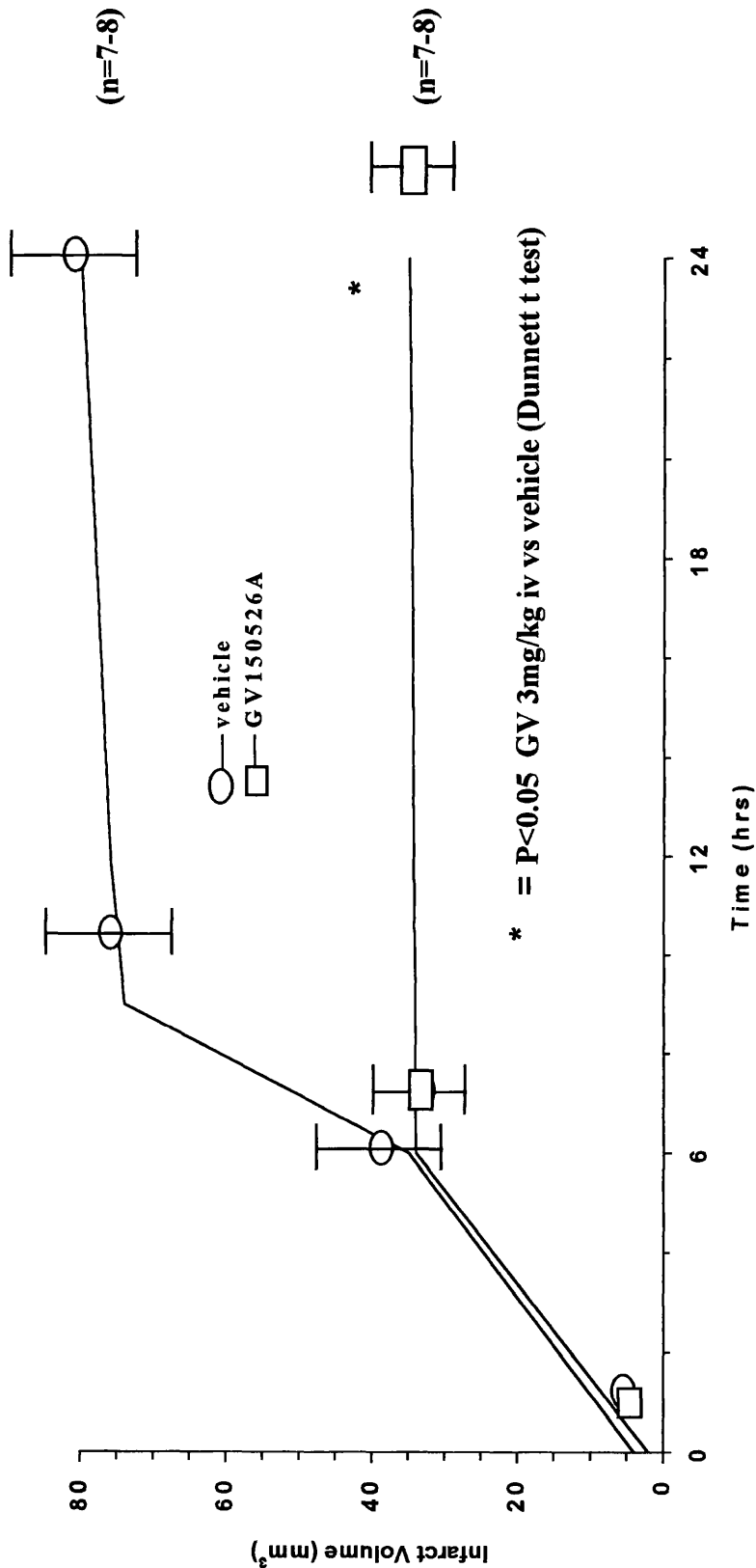


Figure 5.7.4.1: GGT vs time in patients receiving 800mg bolus followed by various bd infusions of GV150526.

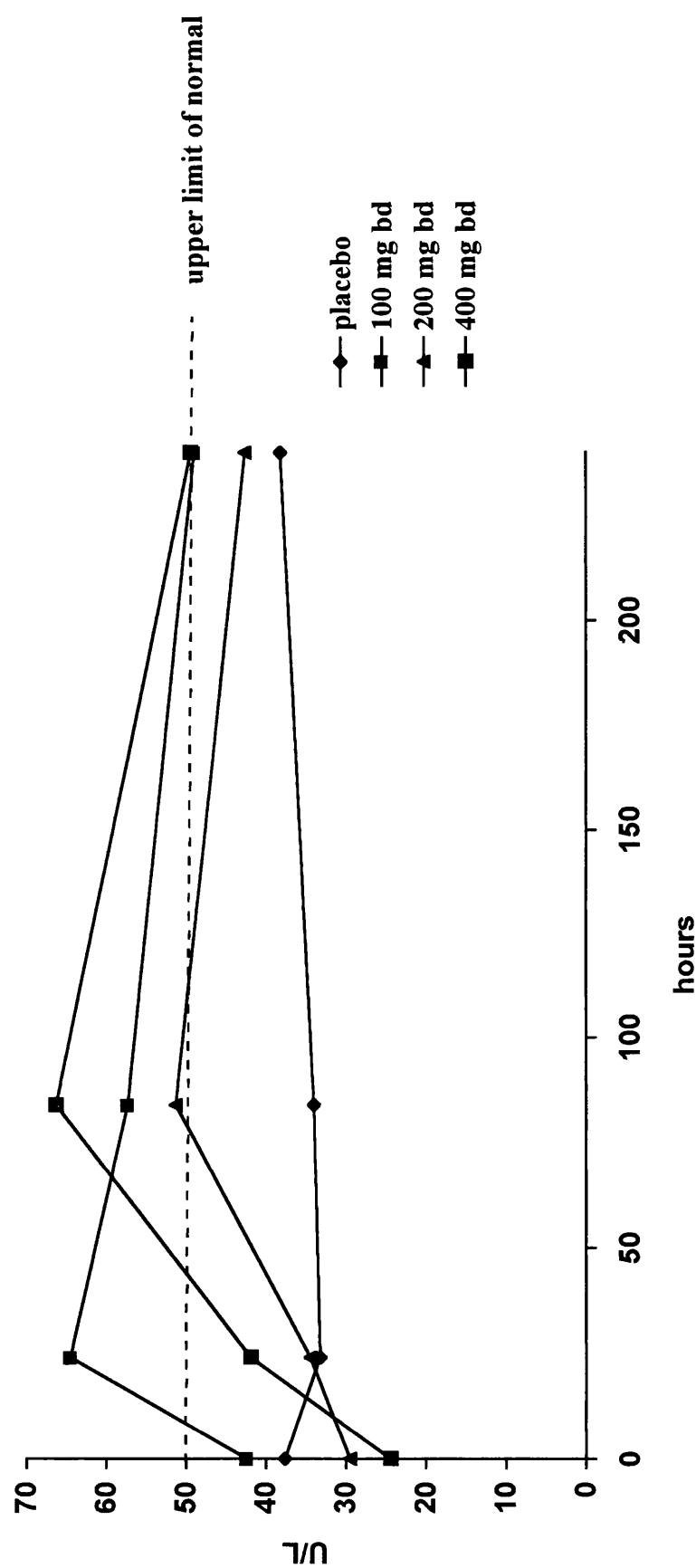


Figure 5.7.4.2: Alkaline phosphatase vs time in patients receiving 800mg bolus followed by various bd infusions of GV150526.

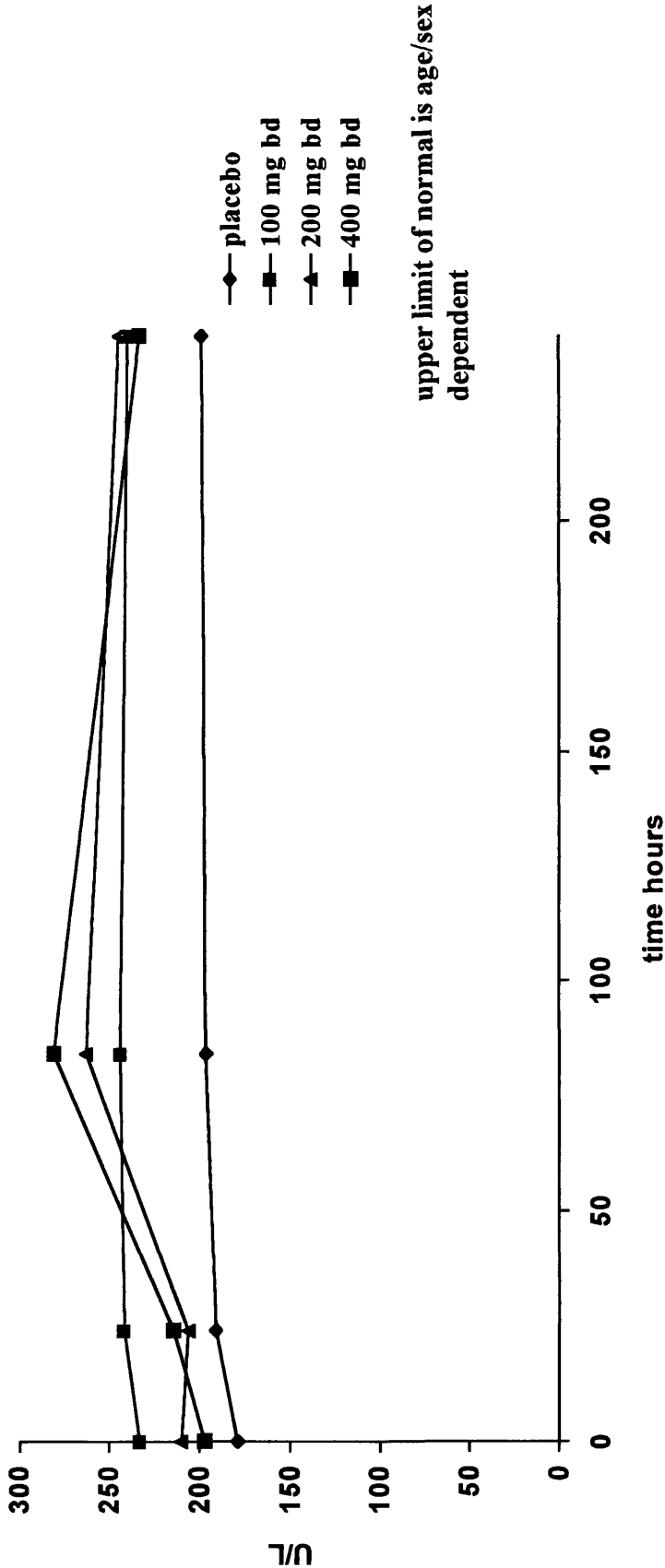


Figure 5.7.7.3: Mean changes in bilirubin vs time in patients receiving 800mg loading dose of GV150526 followed by various ± bd infusion doses.

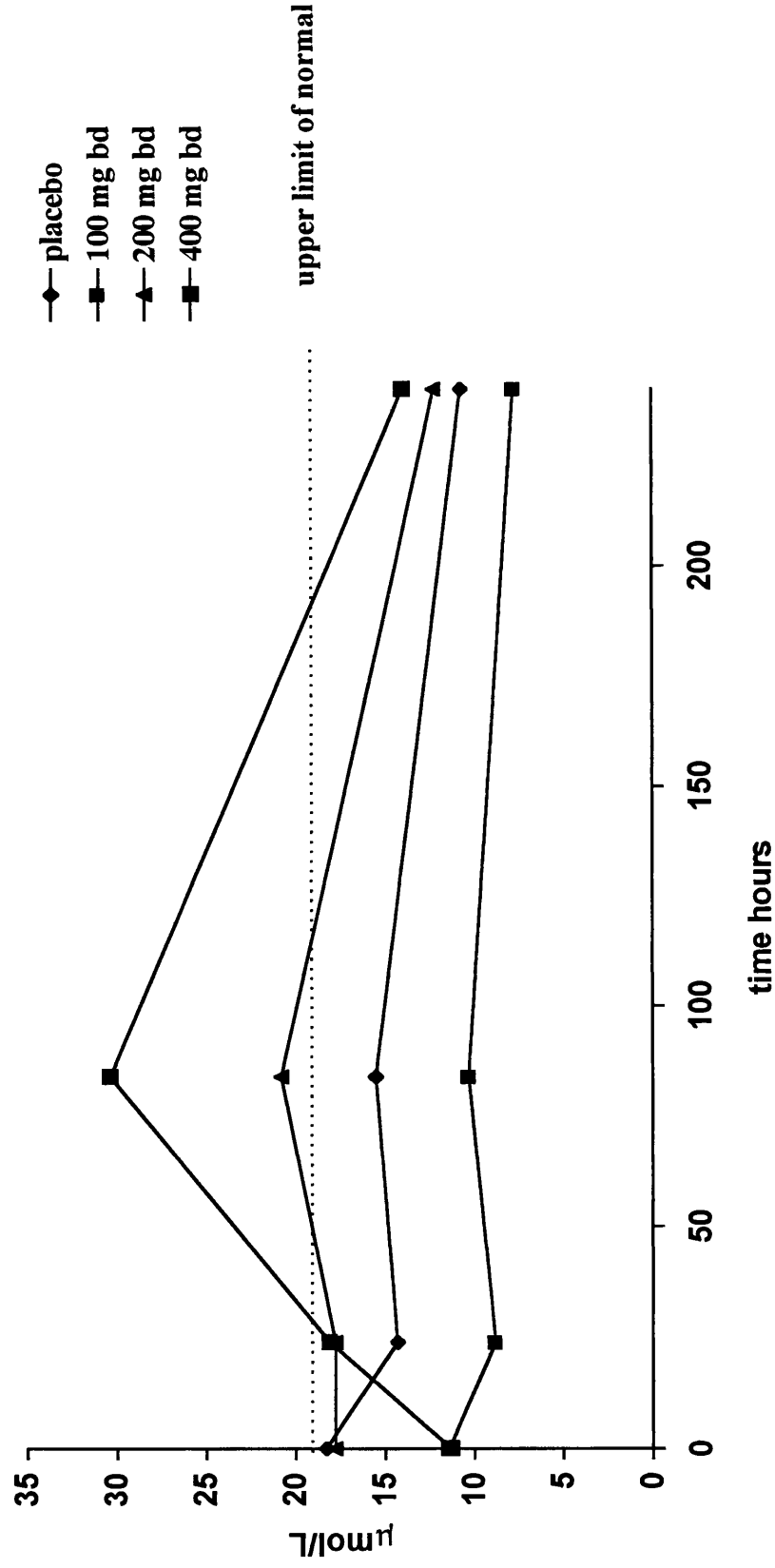


Figure 5.7.4.4: Bilirubin vs time in patients receiving 800mg bolus followed by 400mg bd infusion GV150526.

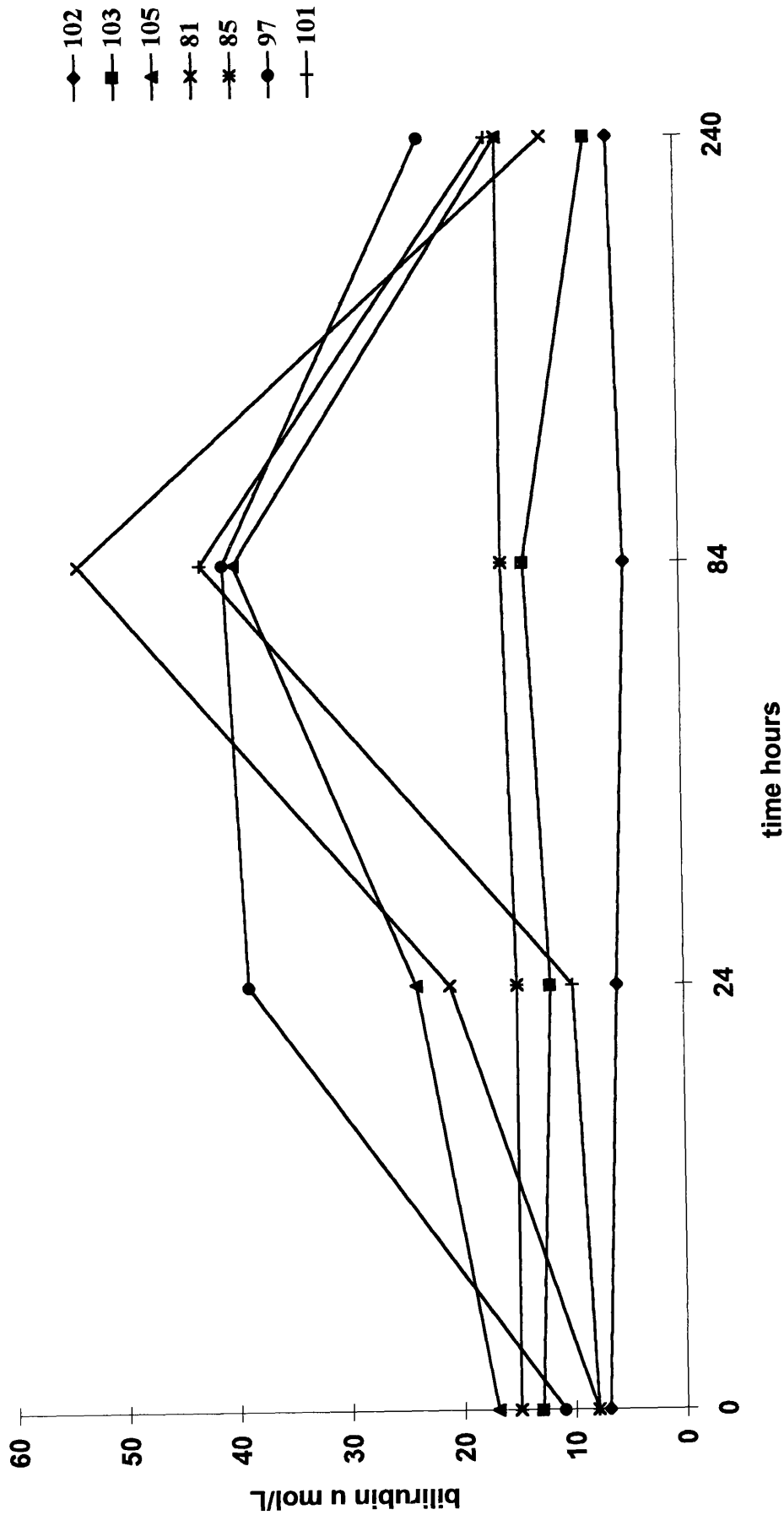


Figure 5.7.4.5: GGT vs time in patients receiving 800mg bolus followed by 400mg bd infuion GV150526.

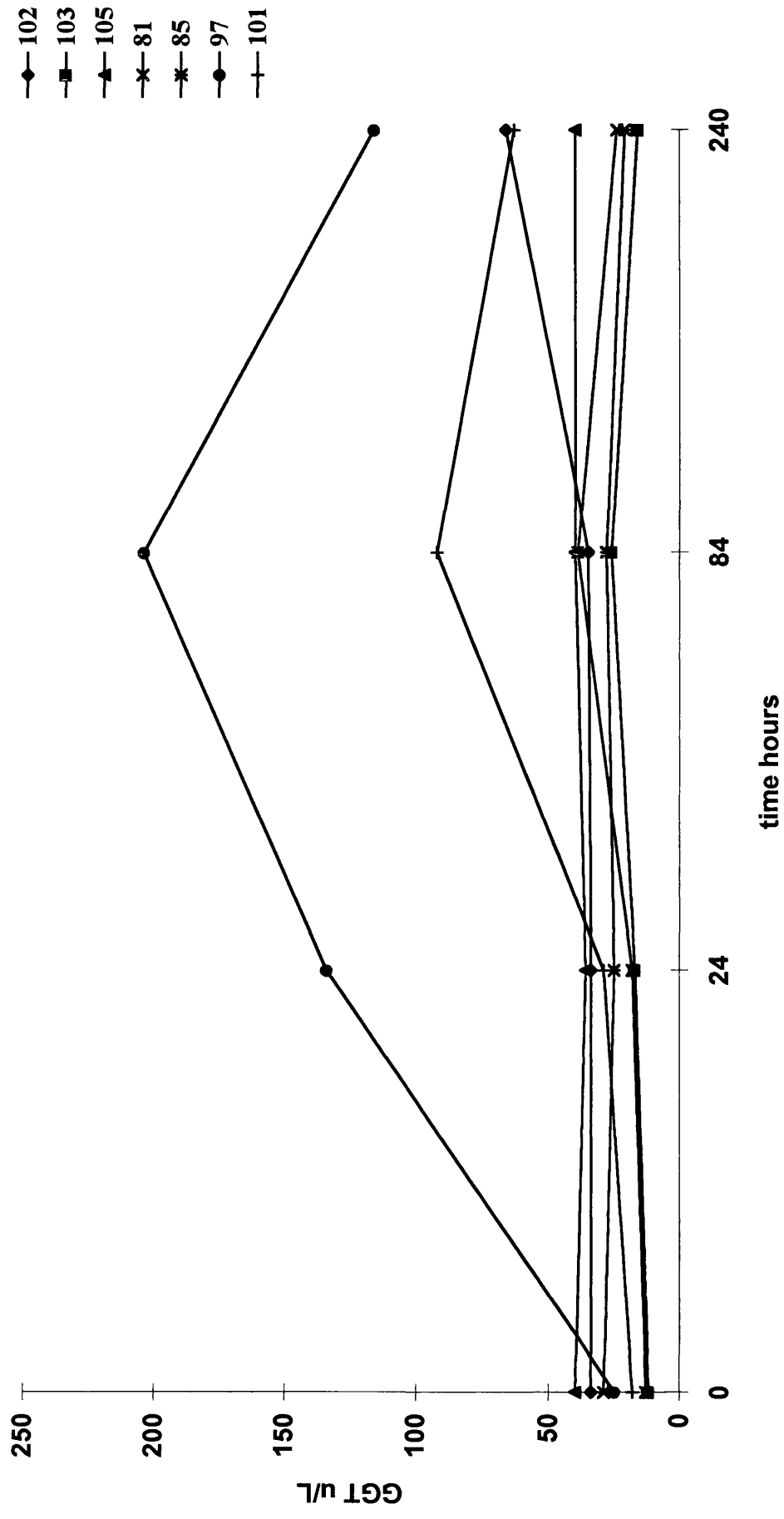


Figure 5.7.4.6: Alkaline phosphatase vs time in patients receiving 800mg bolus followed by 400mg bd GV150526

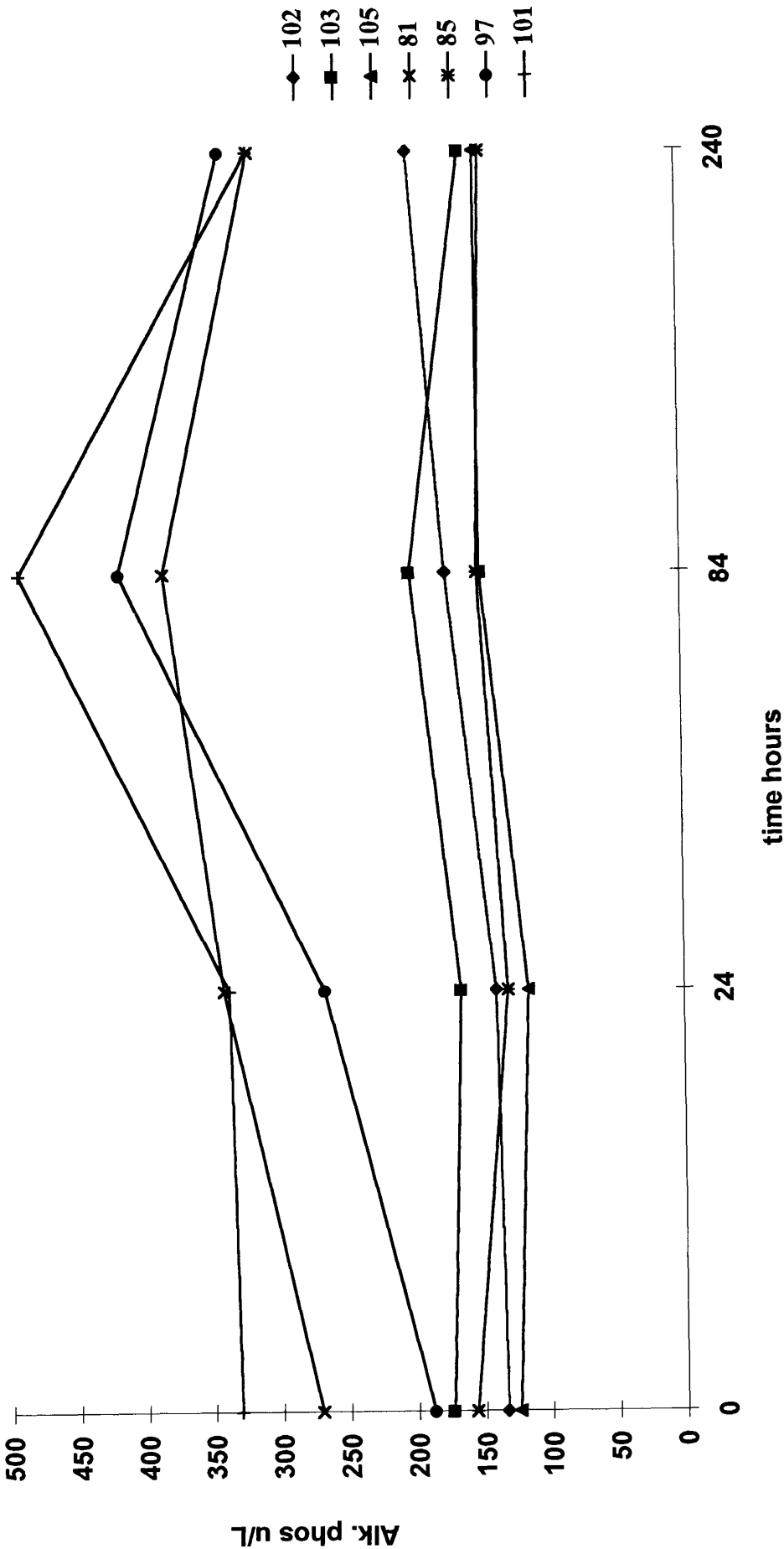


Figure 5.7.6.1: Plasma concentration vs time profile following GV150526 bolus doses

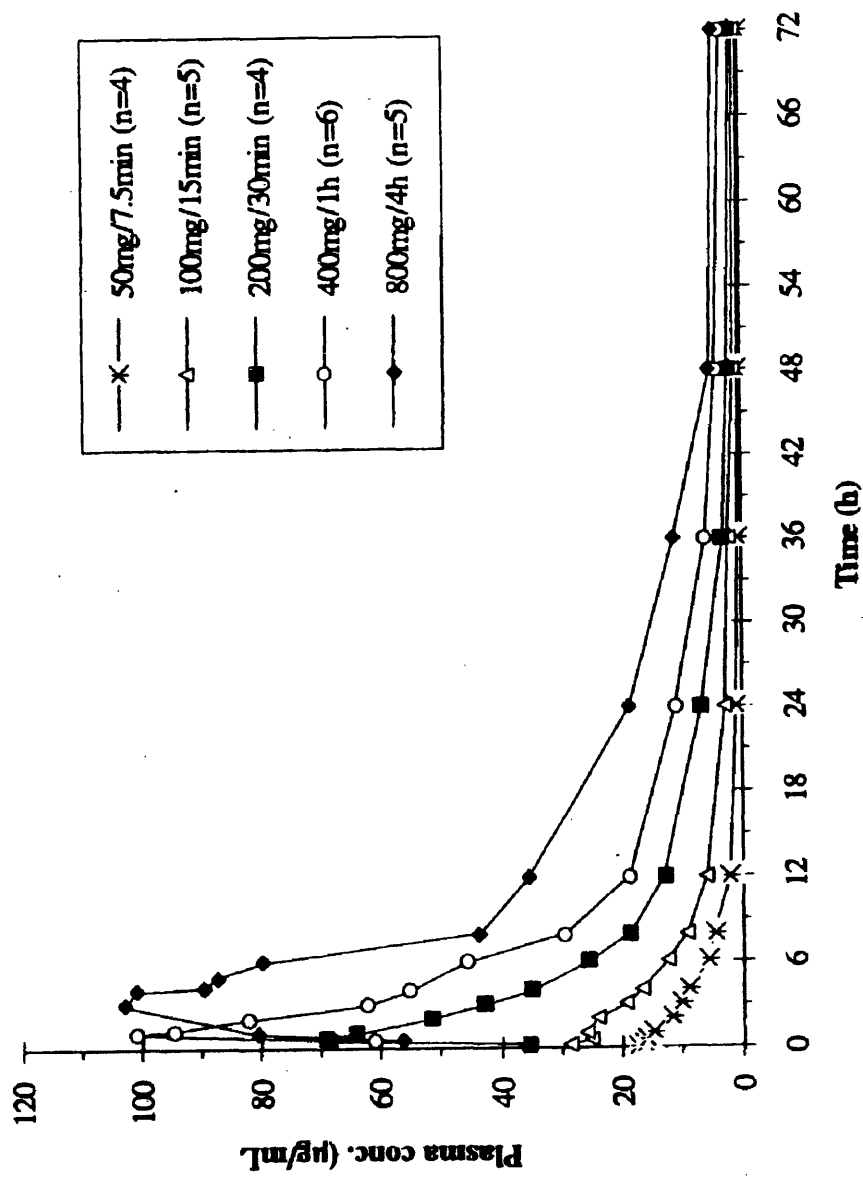


Figure 5.7.6.2: Plasma concentration vs time profile following GV150526 800mg loading dose and 100mg bd x 5

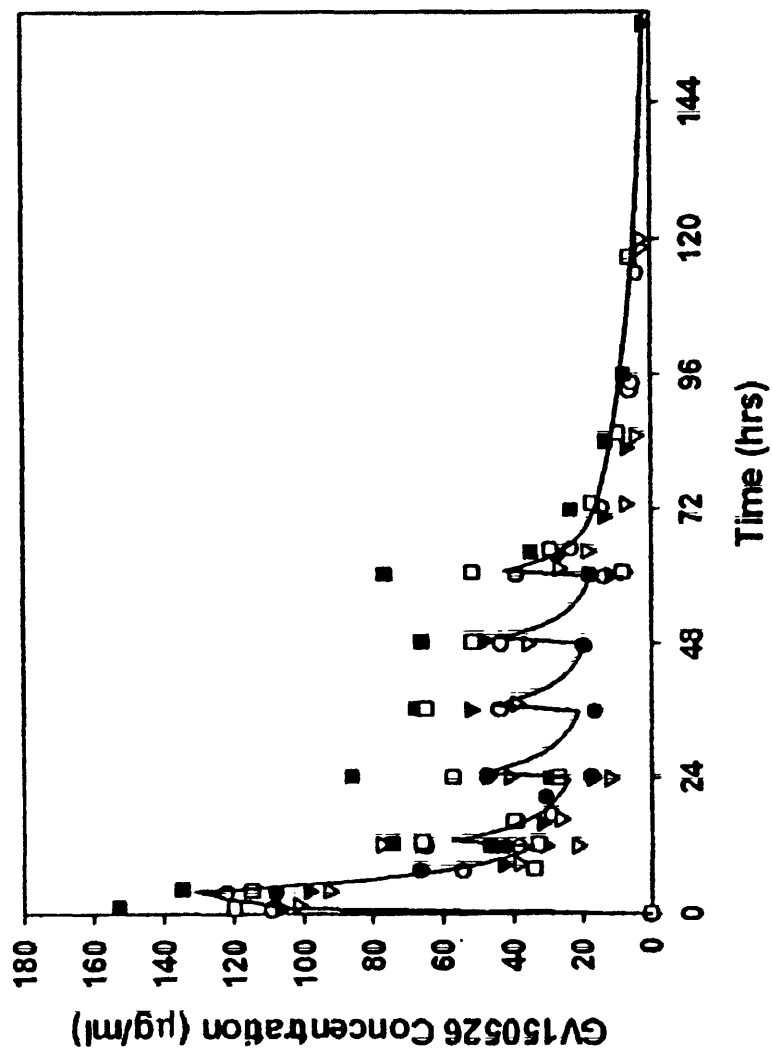


Figure 5.7.6.3: Plasma concentration vs time profile following GV150526 800mg loading dose and 200mg bd x 5

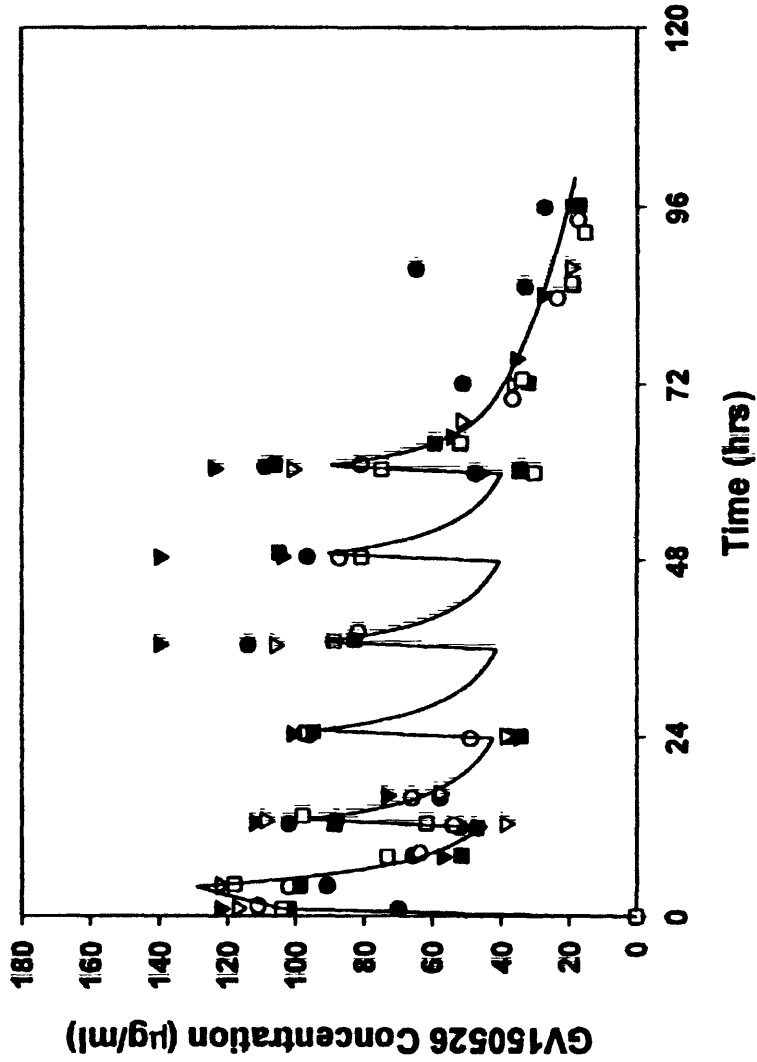


Figure 5.7.6.4: Plasma concentration vs time profile following GV150526 800mg loading dose and 400mg bd x 5

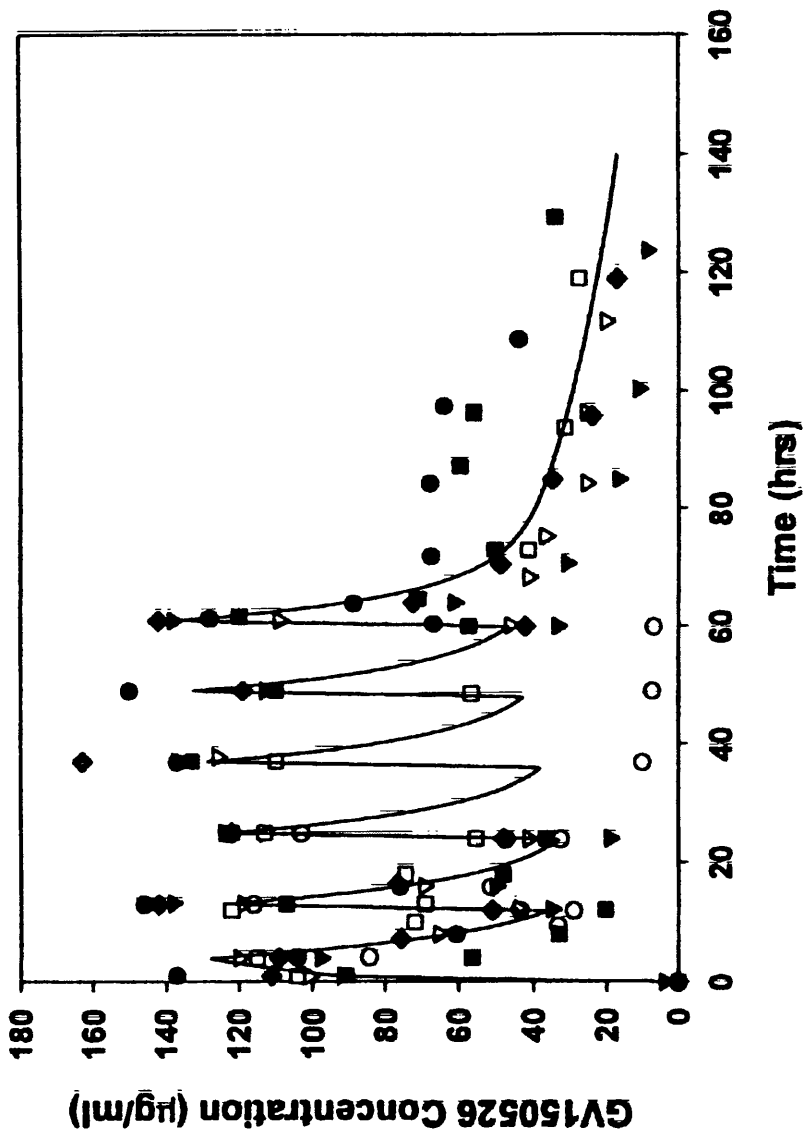


Figure 5.7.7.1: Median NIH Stroke Scale before and 1 month after treatment with placebo or bolus doses of GV150526.

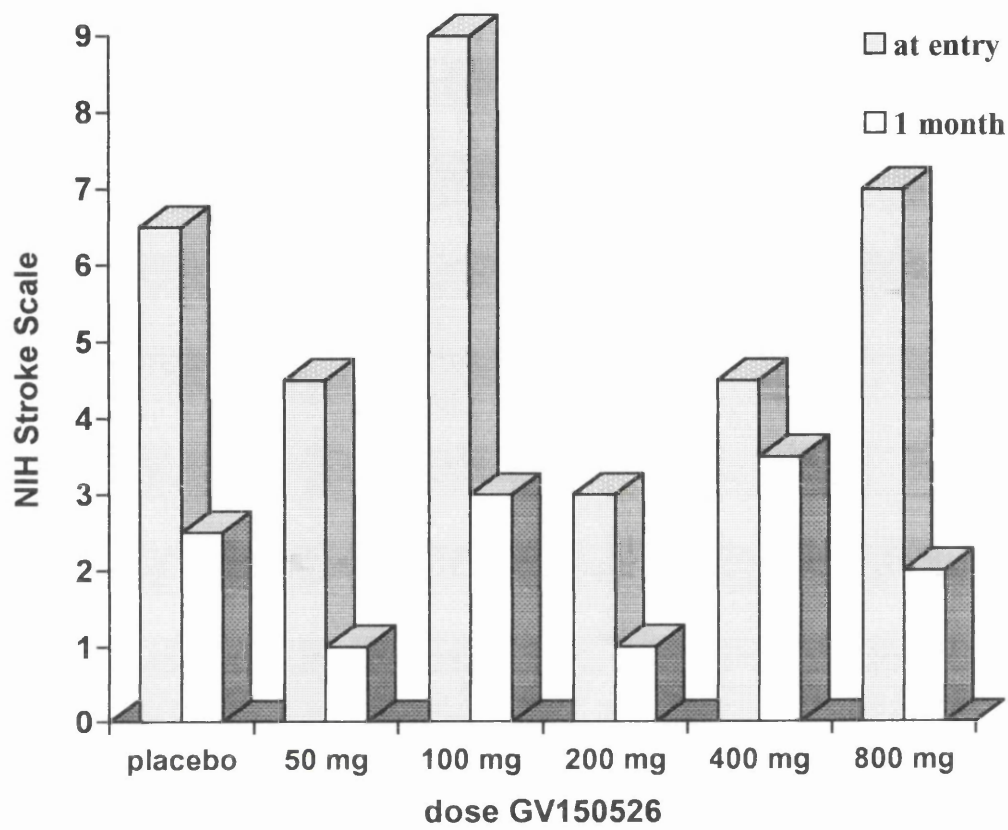


Figure 5.7.7.2: Median Barthel index 1 month after stroke in patients receiving bolus doses of GV150526.

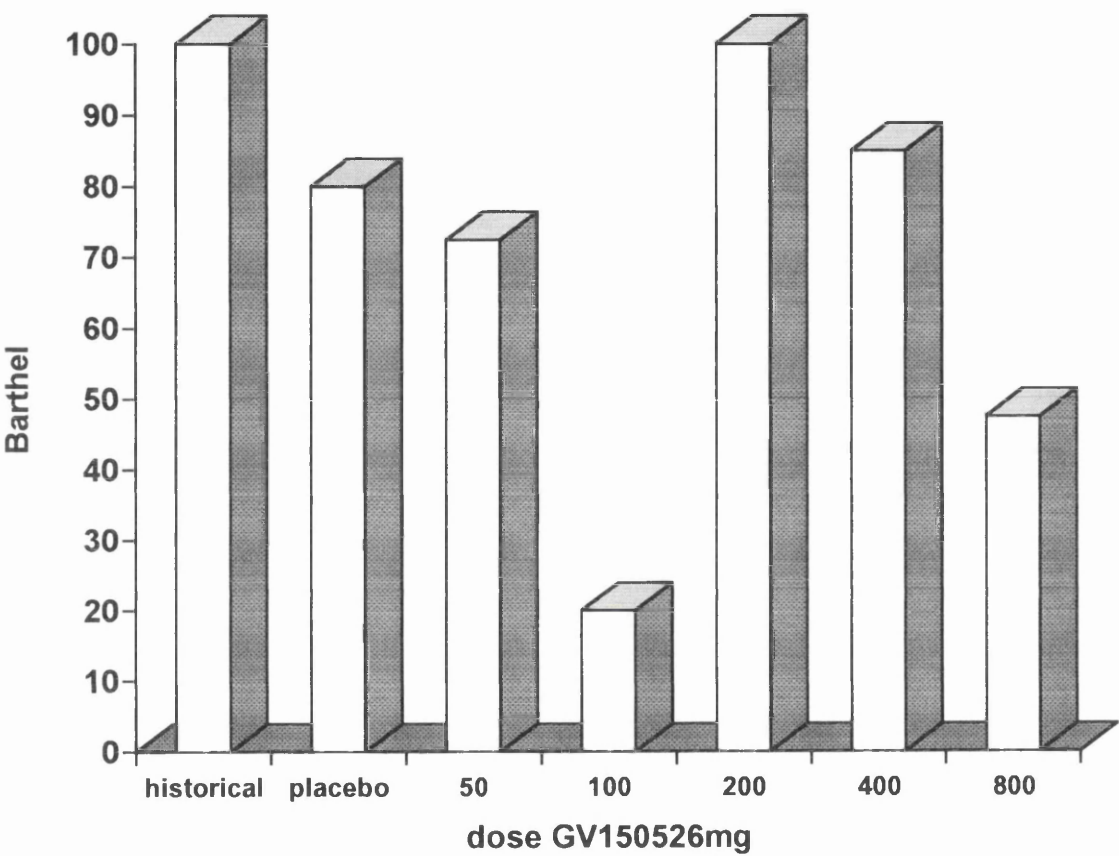


Figure 5.7.7.3: Median NIH Stroke Scale in patients receiving 800mg bolus followed by various doses of GV150526.

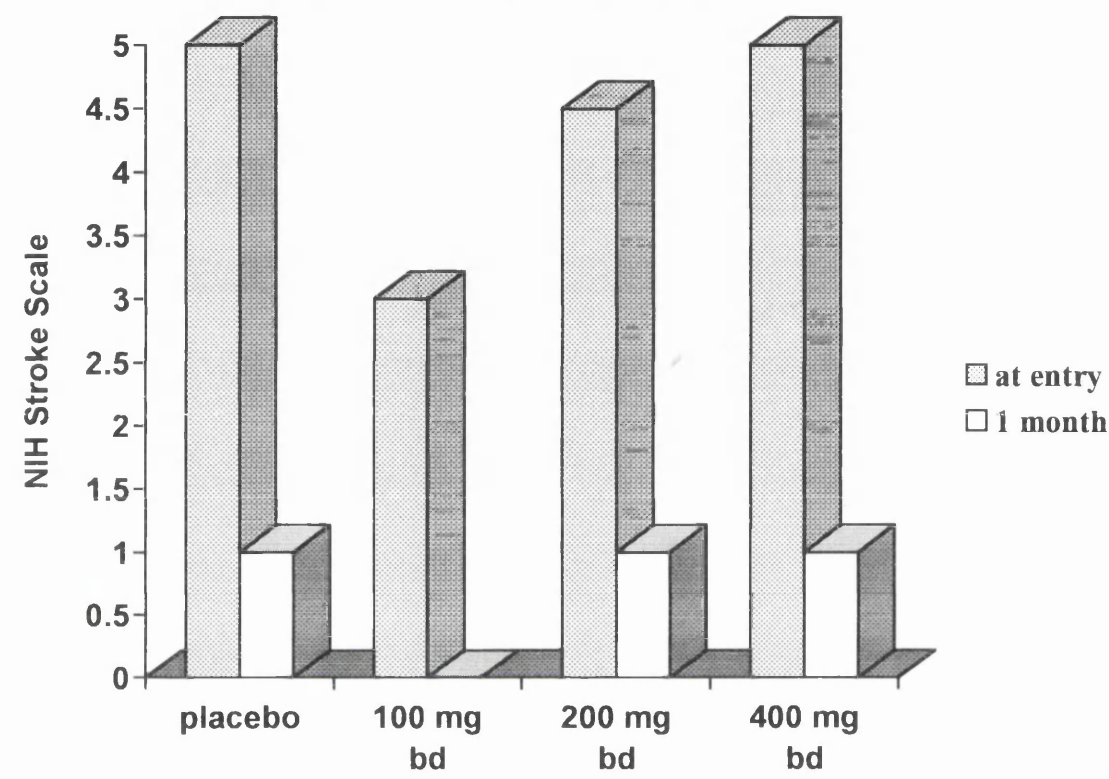
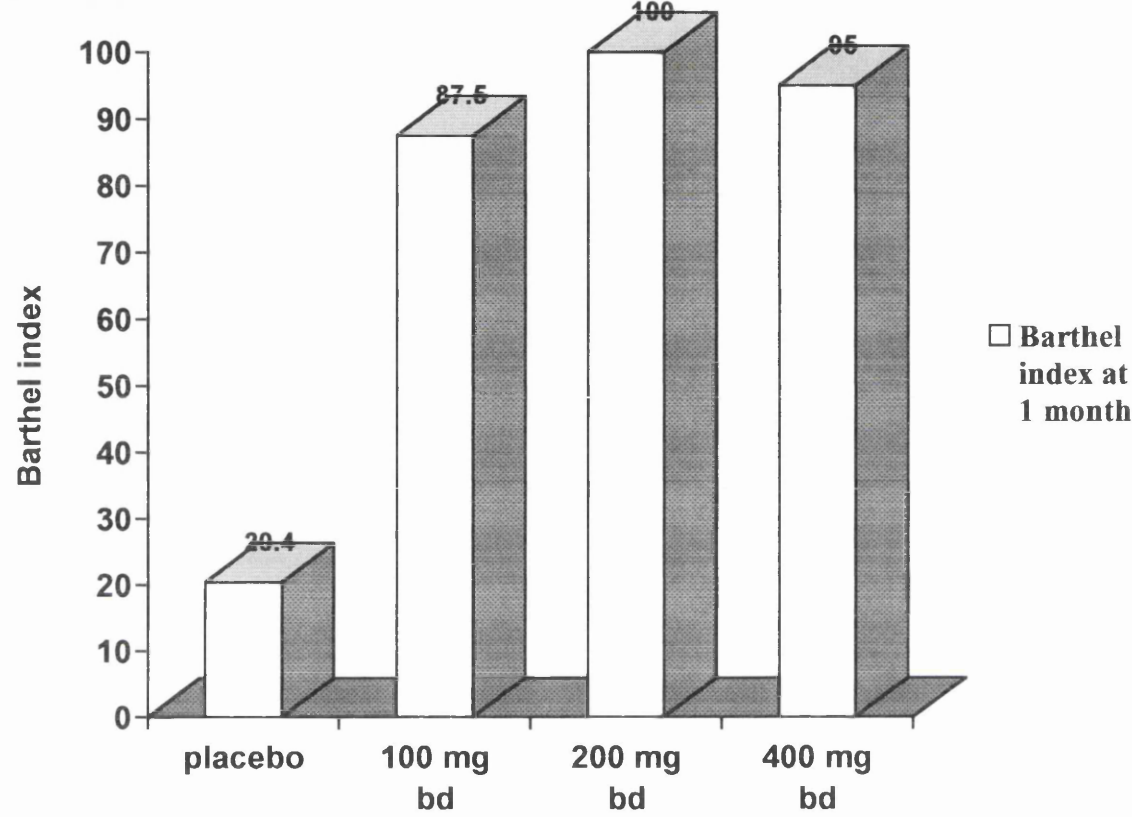


Figure 5.7.7.4: Median Barthel index at 1 month in patients receiving 800mg bolus followed by various bd infusions of GV150526.



Chapter 6

Chapter 6

A phase II double blind, placebo controlled, dose escalation, group comparative study of remacemide hydrochloride in patients with acute ischaemic stroke

6.1 Background

Antagonists of the NMDA receptor inhibit the action of excitatory amino-acids on the post-synaptic neurones and in animal experimental models of stroke significantly reduce the size of cerebral infarcts. Remacemide hydrochloride is a high affinity NMDA antagonist which is currently being evaluated both as a neuroprotective agent and as an anti-convulsant.

6.2 Preclinical pharmacology

Remacemide is an anticonvulsant in animal models and is being evaluated as an anticonvulsant in man. The anticonvulsant and neuroprotective effects have been attributed to its desglycinated metabolite ARL 12495, which is a low affinity antagonist at the ion channel of the NMDA receptor. The structure of remacemide hydrochloride and its desglycinated metabolite are shown in figure 6.2.1.

Remacemide has been shown to be neuroprotective in in-vitro rat cortical neurone cultures exposed to NMDA.²⁴⁵ Rats treated with remacemide after reperfusion in the 4 vessel ischaemia model had smaller infarctions affecting the CA1 and CA3 subregions of the hippocampus. Improvements in pathological findings were matched by improvements in functional outcome (assessed by ability to complete T- maze).¹⁴⁹ Similar results have been obtained in canine models of global ischaemia and in focal MCA occlusion models in rats (Astra unpublished data).

Studies in cats confirm a neuroprotective effect after a focal ischaemic insult. Infusion of remacemide prior to permanent occlusion of the middle cerebral artery reduced the development of infarction in the cortex while the area of dense ischaemia within the caudate nucleus was resistant to neuroprotection.²⁴⁶ The results are summarised in figure 6.2.2. The plasma concentrations required for neuroprotection vary according to the animal model used. The results are summarised in the table below.

model	route	plasma free remacemide	plasma free desglycine metabolite
rat: 4VO	intra-peritoneal	46	64
rat: MCA occlusion: pre-ischaemia	intra-peritoneal	46	64
rat:post-ischaemia	intra-peritoneal	527	106
dog: global ischaemia	iv	681	28
cat: focal	iv	633	95

Preclinical pharmacokinetics suggest remacemide is rapidly absorbed and extensively metabolised with less than 1% excreted unchanged in the urine. In dogs the active desglycinated metabolite crosses the blood brain barrier and the CSF concentration mirrors the plasma concentration.

The toxic effects were similar in mice, rats and rabbits with evidence of alterations in neurological function, particularly seizures and movement disorders. The median lethal dose in rodents for single doses was in the range of 580-993 mg/kg. The maximal tolerated dose in the rat was 160mg/kg. Low incidences of neuronal vacuolation with no evidence of neurone necrosis were reported at this dose.

Haemodynamic effects

Intra-venous infusions of remacemide in conscious beagles produced minor rises in blood pressure and heart rate at doses above 10 mg/kg and this effect was more marked at 30 mg/kg.

In one study of anaesthetised dogs remacemide had no effect on blood pressure at doses of 1,3 and 10 mg/kg. In a second study 1 and 3 mg/kg again had no effect but 10 mg/kg produced a small rise in blood pressure: 10 mmHg between 5 and 15 minutes after administration.

Oral doses of 100mg/kg have no haemodynamic effect on the spontaneously hypertensive rat. The cardiovascular effects described in animals appear mild by comparison with the hypertensive effects of aptiganel described in chapter 7.

6.3 Clinical pharmacology

Volunteer studies suggest good tolerability with this compound. In human volunteers the most common reported side effects were mild CNS disorders e.g.dizziness, headache, mood changes general fatigue and somnolence; or gastro-intestinal symptoms e.g.abdominal pain, dyspepsia, nausea and vomiting.

The clinical pharmacology of remacemide has been investigated in single and multiple dose studies in healthy volunteers and epilepsy patients. Intra-venous single doses (1-300 mg) of remacemide have been given to 19 healthy volunteers with minor adverse events reported only (headache, dizziness and diarrhoea).

Single oral doses (10-500mg) have been given to 68 volunteers within 7 different studies. Remacemide was well tolerated up to 400 mg with dyspepsia, nausea and dizziness reported between 325-400mg. Oral doses of 500mg were poorly tolerated with dizziness, nausea and vomiting reported as the dose-limiting side effects. Multiple oral doses were well tolerated up to 600mg per day. Dose-limiting side effects reported in subjects receiving 125-150 mg four times a day were consistent with those observed in single dose studies.

Pharmacokinetics

Plasma collected from these volunteer studies was used to describe the pharmacokinetics in man. Remacemide was rapidly absorbed orally and eliminated with a half-life ($t_{1/2}$) of 3-4 hrs. Oral remacemide administration demonstrates good bioavailability with a degree of first pass metabolism. The active desglycine metabolite is eliminated more slowly ($t_{1/2}$ 12-18 hrs). The kinetics appear linear with first order elimination. Longer term dosing did not induce hepatic enzymes. Remacemide is concentrated in the brain (brain:plasma ratio 25).

6.4 Aims and objectives

Primary

- The purpose of this study was to assess the safety, tolerability and pharmacokinetics of ascending doses of remacemide in patients with acute stroke.

Secondary

- Neurological and functional outcome data were collected but the power of the study was not powered to demonstrate drug efficacy.

6.5 Patients and methods

6.5.1 Study design

This was a placebo-controlled, escalating dose, single centre parallel group study. Groups of 8 patients (6 active:2 placebo) received treatment, initially consisting of 2 intra-venous infusions, followed by twice daily oral treatment until day 7. Each treatment group was completed and safety data analysed prior to progression to a higher dose group.

Ethics and consent

Ethical approval was gained from the local West Ethics committee. Patients gave written, witnessed informed consent whenever possible. Where patients were unable to write, witnessed verbal consent was accepted from the patient. All witnesses were independent and had no connection with the study. If the patient was unable to give consent verbally written informed assent was accepted from the next of kin or other close family member.

6.5.2 Patient selection

Inclusion criteria

Patients within 12 hours of acute ischaemic stroke were considered for eligibility to the study. NMDA antagonists may be neuroprotective in patients with intra-cerebral haemorrhage and these patients were not excluded from the study.

Other entry criteria to be satisfied are listed below.

- clinical signs of middle cerebral artery occlusion
- male or non pregnant female
- age ≥ 40 and ≤ 85 years
- written or verbal and witnessed informed consent
- capable of attending for follow up as required by protocol
- functionally independent prior to stroke

Exclusion criteria

Pre-clinical data suggest remacemide may cause GI upset, thus patients with peptic ulcer disease were excluded, together with patients on regular NSAIDS or steroids. No information on possible interactions with warfarin was available at the time the study was performed and as warfarin has both a narrow therapeutic index and the potential of serious consequences in inappropriate dosages warfarin was disallowed in the study.

Patients were also excluded from the study if they had symptoms and signs suggestive of other significant neurological disease or other active and clinically significant systemic disease, as follows:

- coma
- previous stroke with residual deficit
- seizures and blackouts
- other serious organic brain disease
- trauma
- psychiatric disease
- chronic hepatic dysfunction
- chronic respiratory disease or pneumonia
- gastro-intestinal disease - including peptic ulceration and G-I bleeding
- connective tissue disease
- malignancy within 5 years
- severe hypertension, valvular heart disease or heart failure
- investigational drug administration within one month

Withdrawals

CT scanning prior to inclusion was not a prerequisite to study entry but patients found to have intra-cerebral haemorrhage were replaced but carried on with study medication and full safety data were collected. Patients had study medication discontinued if CT scanning demonstrated any of the following:

- abscess
- sub-arachnoid haemorrhage
- tumour

6.5.3 Dosing regimen

Drug doses given to individual cohorts are documented in the table below. Only the first cohort received orally administered remacemide: subsequent dosing was exclusively intravenous.

cohort number	iv doses in mg	oral doses
1	100mg over 120mins	twice daily 100 mg
	100mg over 120mins	12 hrly stroke until day 7
2	200mg over 120mins × 6	nil
	every 12 hrs	
3	300 over 120mins × 6	nil
	every 12 hrs	
4	400 over 120mins × 6	nil
	every 12 hrs	
5	500 over 120mins × 6	nil
	every 12 hrs	
6	600 over 120mins × 6	nil
	every 12hrs	

6.6 Study procedures

6.6.1 Screening

Following admission to hospital patients were assessed with regard to the entry criteria. On initial screening the following assessments were made.

- Clinical examination
- Canadian neurological scale²⁴⁷
- Vital signs
- Blood samples :-haematology, clinical chemistry, remacemide level.
- urinalysis
- ECG

Patients with significant abnormalities in any of these parameters suggesting active and clinically significant disease, other than acute stroke, were excluded from entering the study.

6.6.2 Safety

Routine biochemistry, haematology, and urinalysis were carried out by standard laboratory methods. Blood and urine were taken for analysis at presentation, 24 hours following start of drug administration and at 7 ± 2 days. Serious adverse events occurring throughout the study period were continuously documented as they occurred.

Biochemical, and haematological parameters measured are documented in the table below.

Biochemistry	haematology	urinalysis
sodium	total white blood cells	blood
potassium	red cells	protein
glucose	haematocrit	ketones
urea	haemoglobin	glucose
creatinine	platelet count	specific gravity
calcium	prothrombin time	
bilirubin	lymphocytes	
albumin	monocytes	
ALT	eosinophils	
AST		
LDH		
alkaline phosphatase		

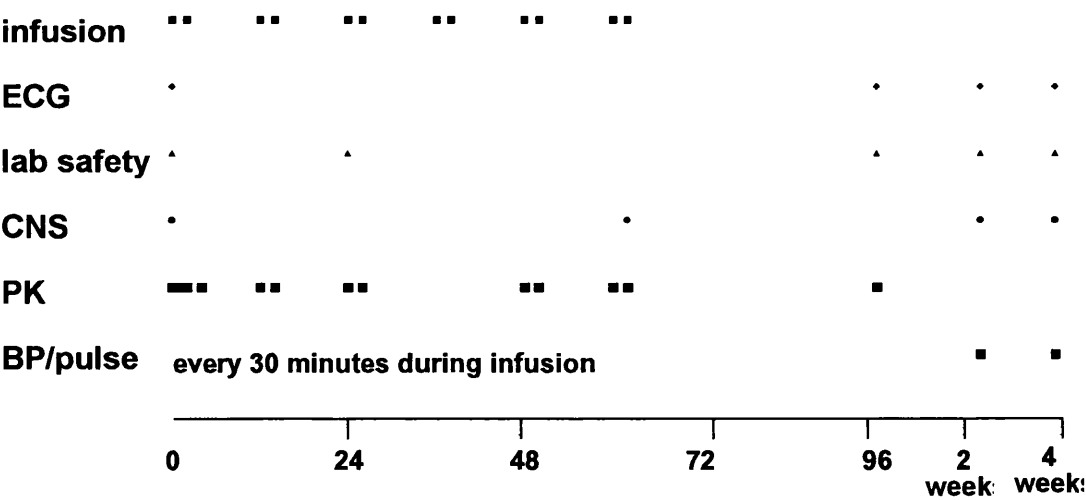
6.6.3 Tolerability

Adverse events were reported as they occurred and at further clinical follow up appointments at 2 weeks and 4 weeks post study entry.

6.6.4 Vital signs

Blood pressure and pulse recordings were made using Marquette semi-automatic oscillometric monitoring equipment (Marquette Electronics Inc, Wisconsin USA) and repeated at frequent intervals during drug dosing as summarised in the plan below.

Protocal summary and study design



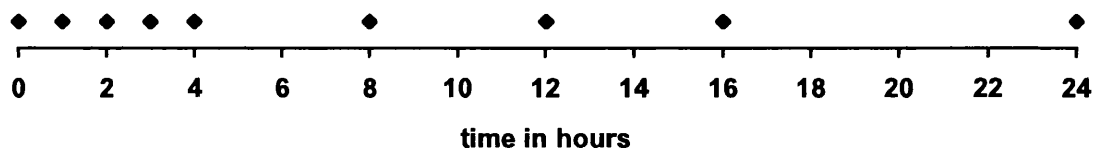
6.6.5 Pharmacokinetics

Blood samples (10 mls) were taken from an in situ iv cannula from the non-infusion arm, collected in a heparinised tube, centrifuged (3000 rpm for 10 mins) and the plasma transfered to a polypropylene tube and frozen immediately thereafter.

Blood was taken for pharmacokinetic (PK) analysis 30 mins and 2 hours after the start of the first infusion. Sampling was repeated at the end of each infusion and before the final infusion.

Times when PK samples were taken are also shown below.

Times in first 24 hours when PK samples taken



Pharmacokinetics were assessed utilising solid phase extraction o-phthalaldehyde derivatisation followed by HPLC separation and fluorescence detection. Plasma was analysed for remacemide and its main desglycine metabolite ARL12495AA. The calibration range of the method is 10- 500 ng/mL for both analyses. Results from samples containing > 500ng/mL were determined after sample dilution in control human plasma to produce a result within the calibration range of the method.²⁴⁸

6.6.6 Outcome

Functional outcome was assessed at the 4 week follow up, using the Barthel functional scale (appendix III) Neurological outcome was assessed by the Canadian neurological scale with assessments made at baseline, 60 hours, 2 weeks and 4 weeks (appendix II).

6.7 Results

6.7.1 Recruitment and demographics

A total of 61 patients were recruited to the study. Forty-three patients completed the treatment course. The demographic details of patients entering the study are shown in the table below. All were caucasian.

Table Patient Demographics

(PRIVATE)Demographics	Placebo bid	100mg bid	200mg bid	300mg bid	400mg bid	500mg bid	600mg bid	All
Number of Patients	18	10	6	8	6	6	7	61
Age (years):Mean	66.6	66.7	65.7	68.4	67.3	67.2	70.4	67.3
Range	49 - 84	46 - 83	45 - 84	41 - 82	46 - 81	60 - 80	55-81	41-84
Sex: Male	7	8	5	6	4	3	3	36
Female	11	2	1	2	2	3	4	25

6.7.2 Compliance

Four patients were withdrawn during the treatment period due to intolerable side effects. A further 10 patients failed to complete the protocol: 2 died during the infusion period(see below), 3 patients were found to have intracerebral tumours on CT scanning and were subsequently withdrawn, 5 patients in the placebo / 100mg dose group developed dysphagia and as a result could not comply with oral medication. The protocol was thereafter amended to allow all doses to be administered via intravenous infusion.

6.7.3 Serious adverse events

Mortality

Two patients died during the study infusion period and a further 7 during the 1 month follow up period. No deaths were considered to be due to administration of study drug in the opinion of the investigator. There was no excess mortality in the remacemide treated patients compared with the placebo group (figure 6.7.3.1).

Placebo deaths

Two patients in the placebo group died. Patient 004A was unable to swallow following a large dominant middle cerebral artery infarction and therefore only received 2 iv bolus doses. Two days later the patient's level of consciousness deteriorated and never fully recovered: the subject died 11 days later. The clinical deterioration was in keeping with the CT findings which confirmed a large infarction with considerable peri-infarction oedema and mass effect.

Patient 4 died 6 days after receiving remacemide. The patient had been admitted with a large cerebral infarction with complicating raised intracranial pressure. The patient received iv drug only. As she never regained consciousness she was unable to comply with oral medication.

100 mg dose

- Two out of ten patients in the 100 mg dose group died within 1 month of study drug administration: patient 001 received 2 iv doses only as the patient was unable to swallow. CT scan demonstrated an intracerebral haemorrhage and the patient was withdrawn from the study. The patient was transferred to another hospital but died following an episode of bronchopneumonia.
- Patient 007 died following a massive pulmonary embolism which occurred 3 weeks after administration of study drug. CT scan had diagnosed an underlying brain tumour.

200 mg dose

Two of the six patients in the 200 mg dose group died.

- Patient 010 suffered a sudden cardiorespiratory arrest 4 days after the administration of study drug. The cause of death was thought to be pulmonary thrombo-embolism.
- Patient 016 died on the day after admission as a consequence of a large cerebral infarction with raised intracranial pressure and mass effect confirmed on CT. Drug treatment was discontinued after 2 iv doses because of the deterioration in the patient's condition.

300 mg dose

One from a total of 8 patients (024) died 2 days after admission following 4 iv doses.

Although the death occurred during the infusion there was no evidence to suggest this was a drug effect. The cause of death was transtentorial herniation secondary to a large cerebral infarction.

400 mg dose

One from six patients (025) died 7 days after the final administration of study drug. The cause of death was a combination of congestive cardiac failure and bronchopneumonia.

500 mg dose

Patient 034 died of congestive cardiac failure 3 days after the last dose of study therapy.

No deaths were thought to be attributable to drug therapy. The mortality rate for those receiving placebo was considerably lower than expected at 6% whereas active treatment was consistent with known previous experience at 16%. There was no excess of deaths at the higher doses of remacemide, compared with lower doses.

Non fatal adverse events**Withdrawals**

Four patients given active treatment and one placebo patient were withdrawn after developing side effects attributed to drug therapy. One patient in the 200 mg group was withdrawn for an infusion site reaction (pain, erythema and swelling). A patient was withdrawn after 500 mg with CNS adverse effects (hyperreflexia and agitation) and a further 2/7 patients withdrew from the highest 600 mg bd dose with CNS adverse effects: hallucinations, nausea and vomiting in one case and dizziness, sedation and vomiting in the

other. Two patients in the first dose cohort were withdrawn from the study due to dysphagia. This prevented further compliance with the protocol, which was subsequently amended.

Patient withdrawals are summarised in the table below.

patient	treatment	doses received	reason for withdrawal
4	placebo	2 iv	dysphagia: patient died
4A	placebo	2 iv	dysphagia: patient died
6	placebo	2 iv + 5 oral	brain tumour on CT
6A	placebo	1 iv	dysphagia
24	placebo	4 iv	died due to stroke: day 4
38	placebo	5 iv	confused *
1	100 mg bd	2 iv	dysphagia
1B	100 mg bd	2 iv	dysphagia
2	100 mg bd	2 iv + 4 oral	brain tumour
7	100 mg bd	2 iv + 1 oral	brain tumour
11	200 mg bd	4 iv	intolerance: infusion site reaction *
16	200 mg bd	2 iv	died due to stroke: day1
19	300 mg bd	1 iv	diagnosis not stroke
21	300 mg bd	1 iv	CT = haemorrhage: withdrawn in error
37	500 mg bd	5 iv	intolerance: hyperreflexia and agitation *
43	600 mg bd	2 iv	transferred from ward for emergency surgery
43A	600 mg bd	4 iv	intolerance: hallucinations, nausea and vomiting *
44	600 mg bd	2 iv	intolerance: light headed, sleepy and vomiting *

* Patients withdrawn due to drug attributed adverse events.

Serious adverse events

Reassuringly there was no increase in the number of serious adverse events reported at the higher doses of remacemide (figure 6.7.3.2).

Vital signs

There was no difference in vital signs (heart rate, blood pressure) and no excess of ECG abnormalities in patients receiving remacemide compared with placebo.

Other adverse events

There was an increase in reported CNS adverse events and gastro-intestinal effects at the higher doses of 400, 500 and 600 mg (figures 6.7.3.3, and 6.7.3.4). The most common treatment attributed adverse events were neurological. Infusion site reactions and gastro-intestinal upset, particularly nausea and vomiting were also reported. CNS and gastro-intestinal events adverse events were frequent in the group receiving 600 mg infusions. CNS events increased in frequency during later infusions in the 500 and 600 mg dose groups consistent with accumulation of either remacemide or its active metabolite (figure 6.7.3.5).

adverse event	placebo	active	description
	n=18	n=43	
infusion site reaction	0	17	
neurological	0	5	headache, hyperreflexia, diplopia, dizziness tremor
psychiatric	1	6	3x agitation, 2x confusion, 2x hallucinations sedation
gastro-intestinal	1	4	4x nausea and vomiting

NB. Some patients reported more than one symptom

6.7.4 Laboratory results

There was no increase in the frequency of abnormal laboratory reports within the patients treated with remacemide compared with placebo.

6.7.5 Pharmacokinetics

Pharmacokinetics indicate a short half life of 3-4 hours for remacemide but a more prolonged half life (12-18 hours) for its desglycinated metabolite (ARL 12495AA).

Pharmacokinetic analysis demonstrated no accumulation of remacemide during the course of repeated infusions at the higher doses but did reveal accumulation of the active desglycine metabolite. This suggests the active metabolite is responsible for the onset of

CNS and gastro-intestinal effects after repeated infusions of remacemide (figures 6.7.5.1 and 6.7.5.2).

6.7.6 Clinical outcome

There was no significant difference between neurological and functional outcome between the remacemide and placebo receiving groups. Results are summarised in figures 6.7.6.1 and 6.7.6.2.

6.8 Discussion

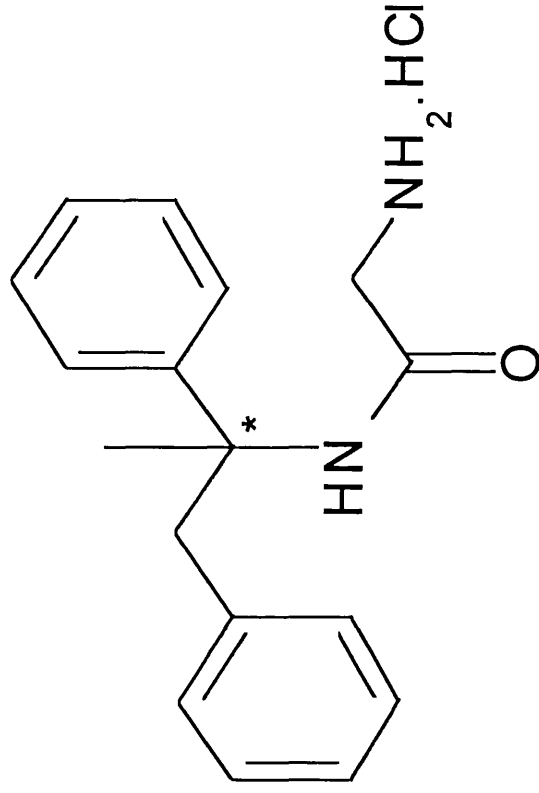
Doses of 200 mg bd or higher attained the putative neuroprotective concentrations predicted from animal models (250-600ng/mL). Doses of 500 mg bd were well above these target levels with an acceptable tolerability profile (only one patient was withdrawn due agitation and hyperreflexia). No patients were withdrawn from the 400 mg dose group. Doses of 600 mg bd iv were associated with an unacceptable side effect profile. 2/7 patients at this dose required discontinuation of study drug due to intolerable CNS side effects. Side effects reported at this dose were hallucinations, nausea and vomiting in one individual and sedation, nausea and vomiting in another.

Side effects, particularly hallucinations, agitation and vomiting were thus reported at the higher doses and particularly during the later infusions. These findings would suggest an accumulation of either remacemide or active metabolite over time at higher doses. The desglycine metabolite accumulated during the course of repeated infusions consistent with the clinical findings. Thus, if remacemide was administered over a more prolonged duration i.e.greater than 72 hours, the maximum tolerated dose may be lower than was observed within this study.

Whether the side effects would preclude therapeutic administration of remacemide is difficult to assess in the absence of phase III efficacy data. Clearly if treatment was moderately effective in improving neurological and clinical outcome, unpleasant but temporary side effects would be acceptable. An analogous example is the use of radiotherapy and chemotherapy in the treatment of cancer, where short term side effects are often unpleasant but considered acceptable because of the poor prognosis of the untreated condition.

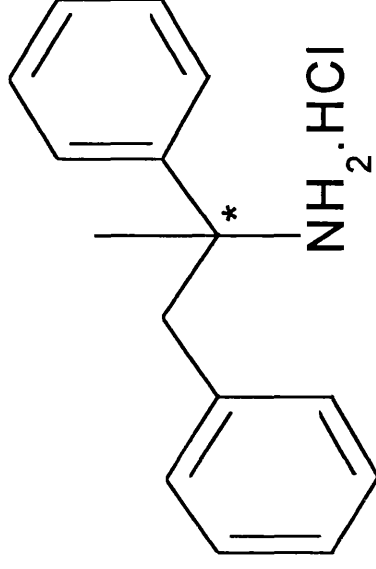
The maximum tolerated dose for use in acute stroke is 400 mg bd. The gradual accumulation of active metabolite and the gradual onset of adverse events suggests that optimal neuroprotective concentrations are unlikely to be achieved within the early hours of treatment at this dose. The most conclusive evidence of neuroprotective effect from the cat focal ischaemia model was obtained by pretreatment with remacemide.²⁴⁶ For this reason, remacemide may not be an ideal drug to pursue for acute neuroprotective studies.

Figure 6.2.1: Structure of remacemide and desglycinated metabolite



Remacemide hydrochloride

(ARL 12924AA)



desglycinated metabolite

(ARL 12495AA)

Figure 6.2.2: Neuroprotective effect of remacemide in cat focal middle cerebral artery occlusion model.
Volume of infarction (mean \pm sem) was measured histologically 6 hours after the onset of ischaemia.

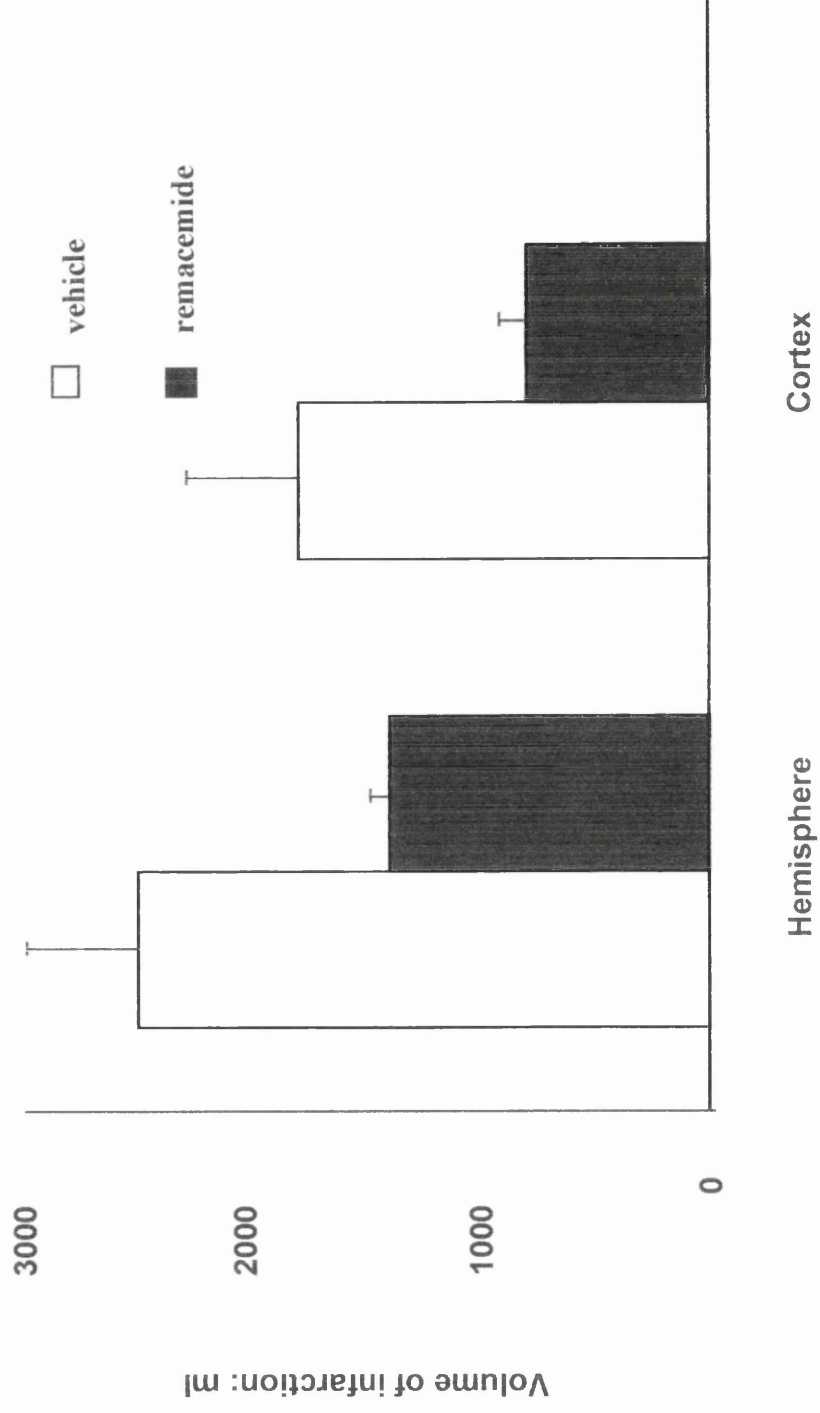


Figure 6.7.3.1: Deaths by 4 weeks in patients treated with remacemide or placebo.

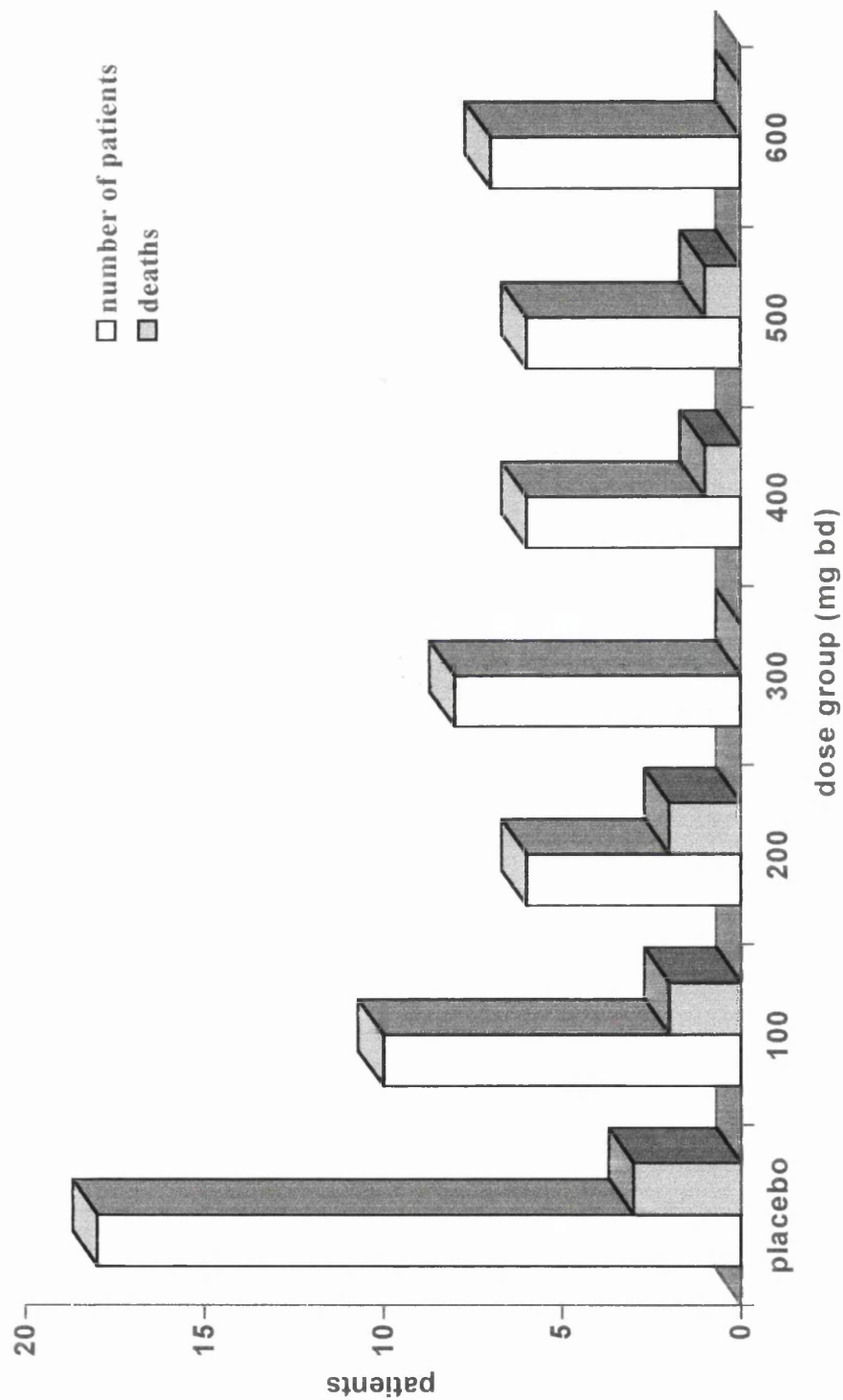


Figure 6.7.3.2: Adverse and serious adverse events reported during drug infusion in patients receiving remacemide

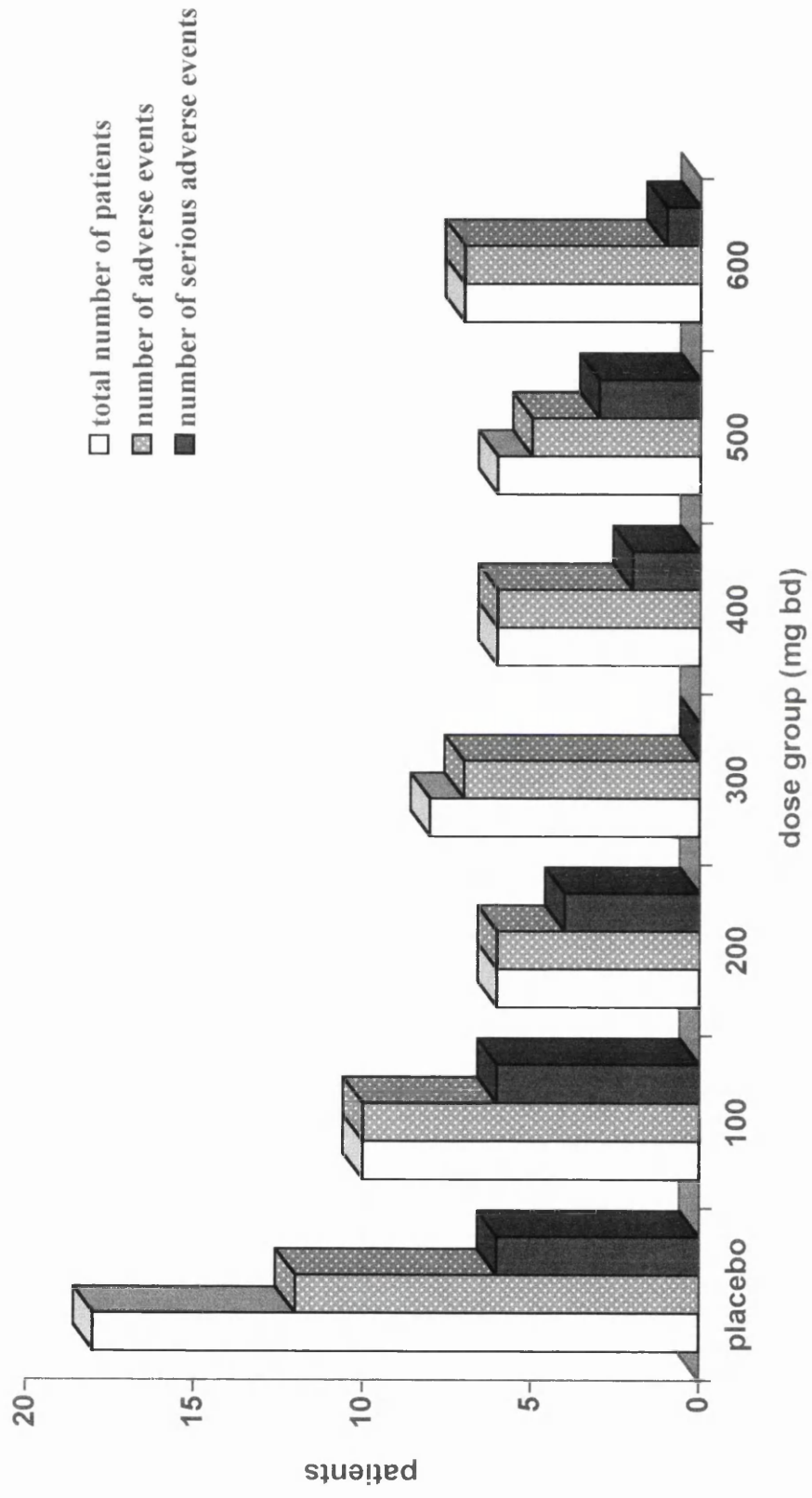


Figure 6.7.3.3: Treatment-attributed CNS events occurring during infusion with remacemide

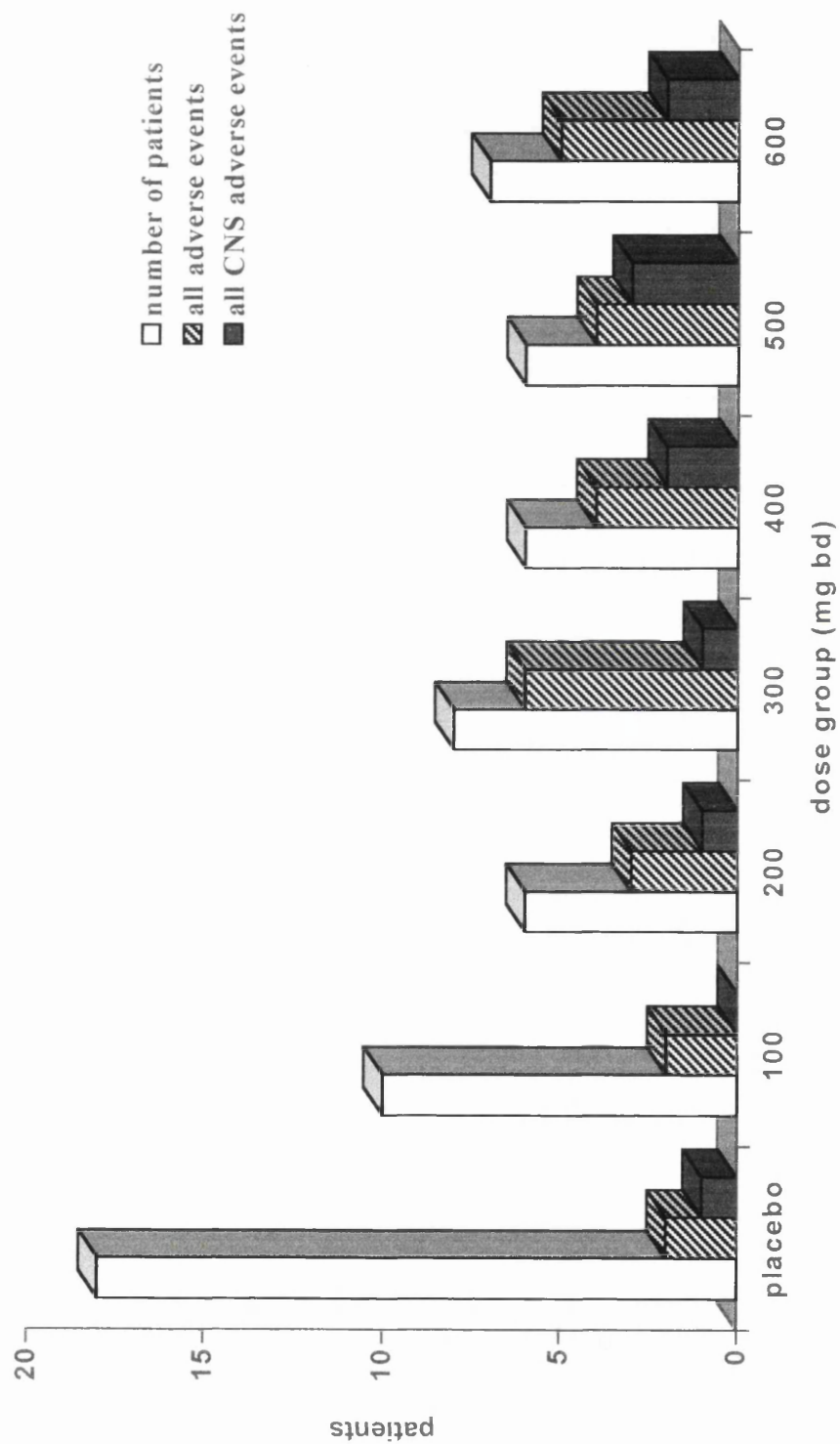


Figure 6.7.3.4: Gastro-intestinal adverse events reported during infusion with remacemide

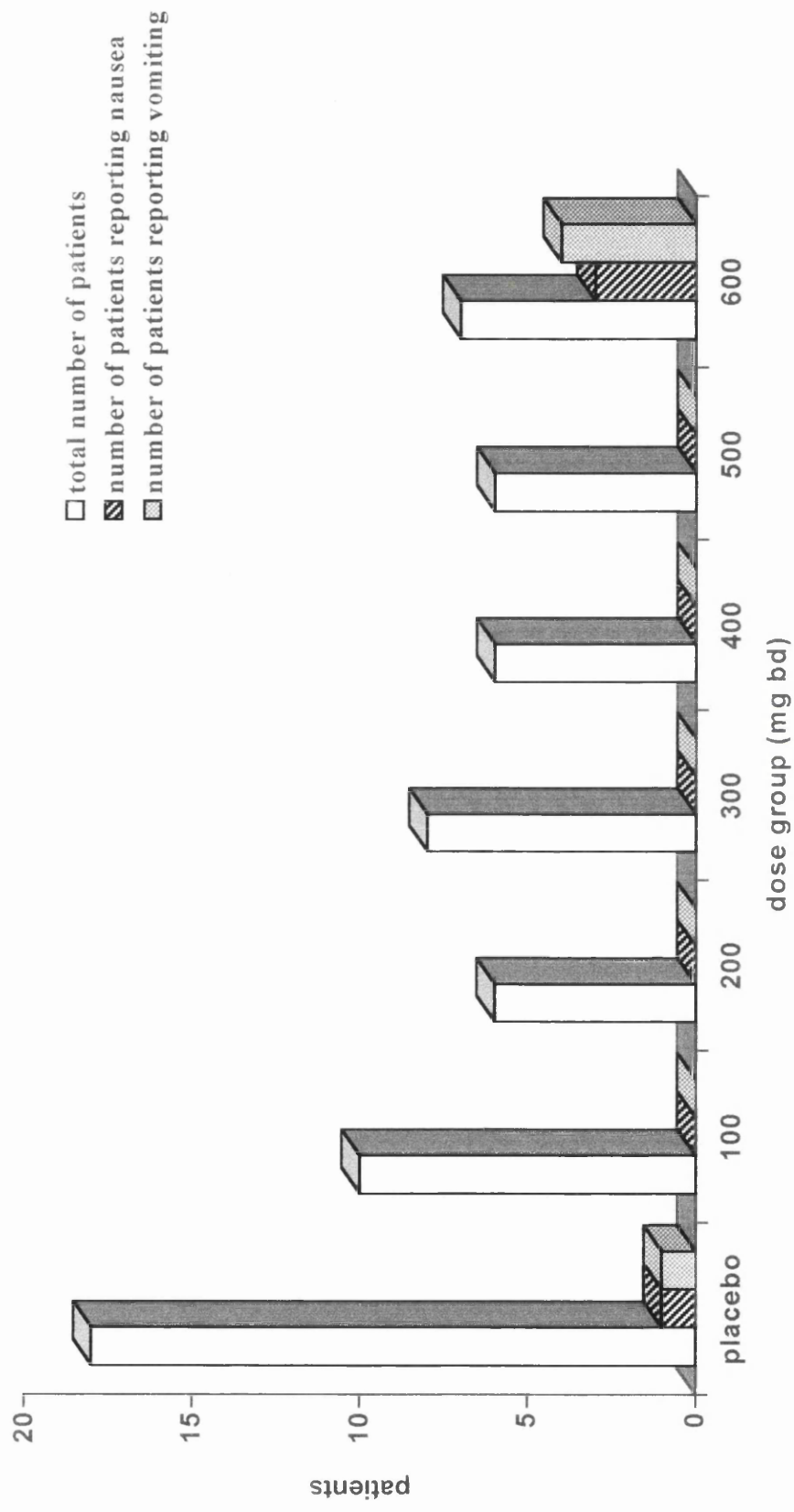


Figure 6.7.3.5: Cumulative CNS events *versus* duration of treatment with remacemide

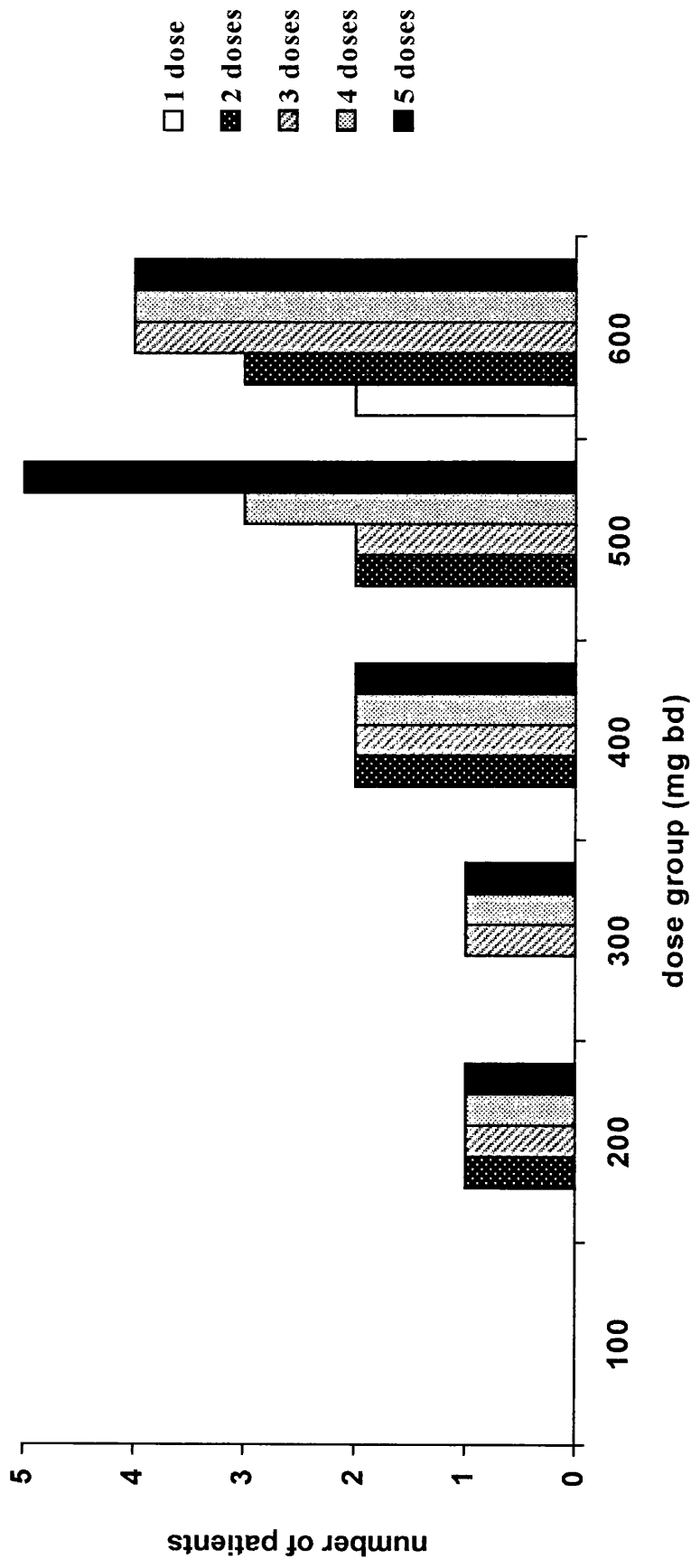


Figure 6.7.5.1 : Concentrations of remacemide (mean \pm sem) . Graph compares plasma concentrations of remacemide after second infusion and after sixth and final infusion. No accumulation of remacemide at higher doses was demonstrated.

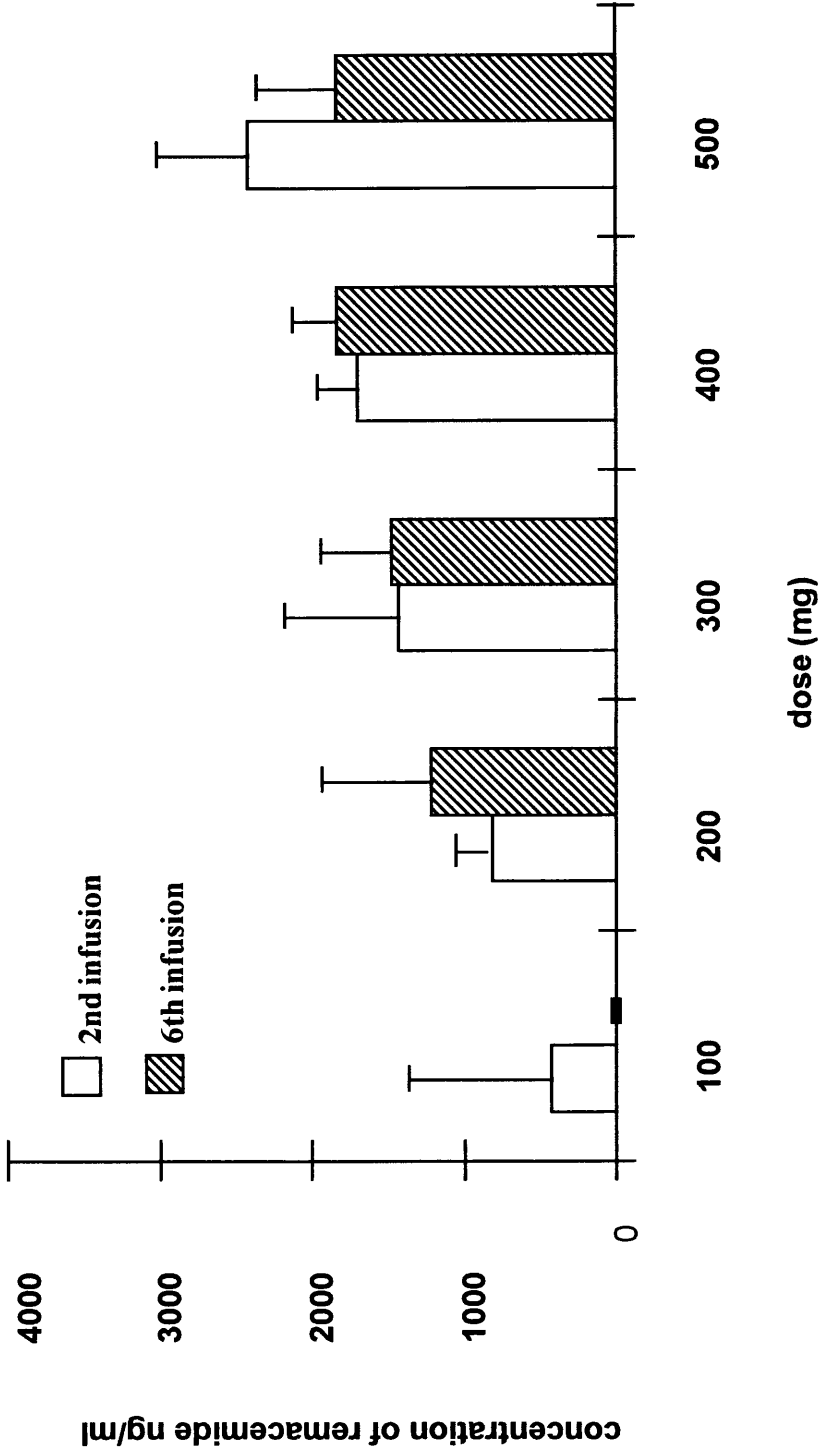


Figure 6.7.5.2 : Plasma concentration (mean \pm sem) of remacemide desglycine metabolite comparing concentration at the end of second infusion with concentration at end of sixth and final infusion. Graph demonstrates accumulation of desglycine metabolite at the end of infusion period.

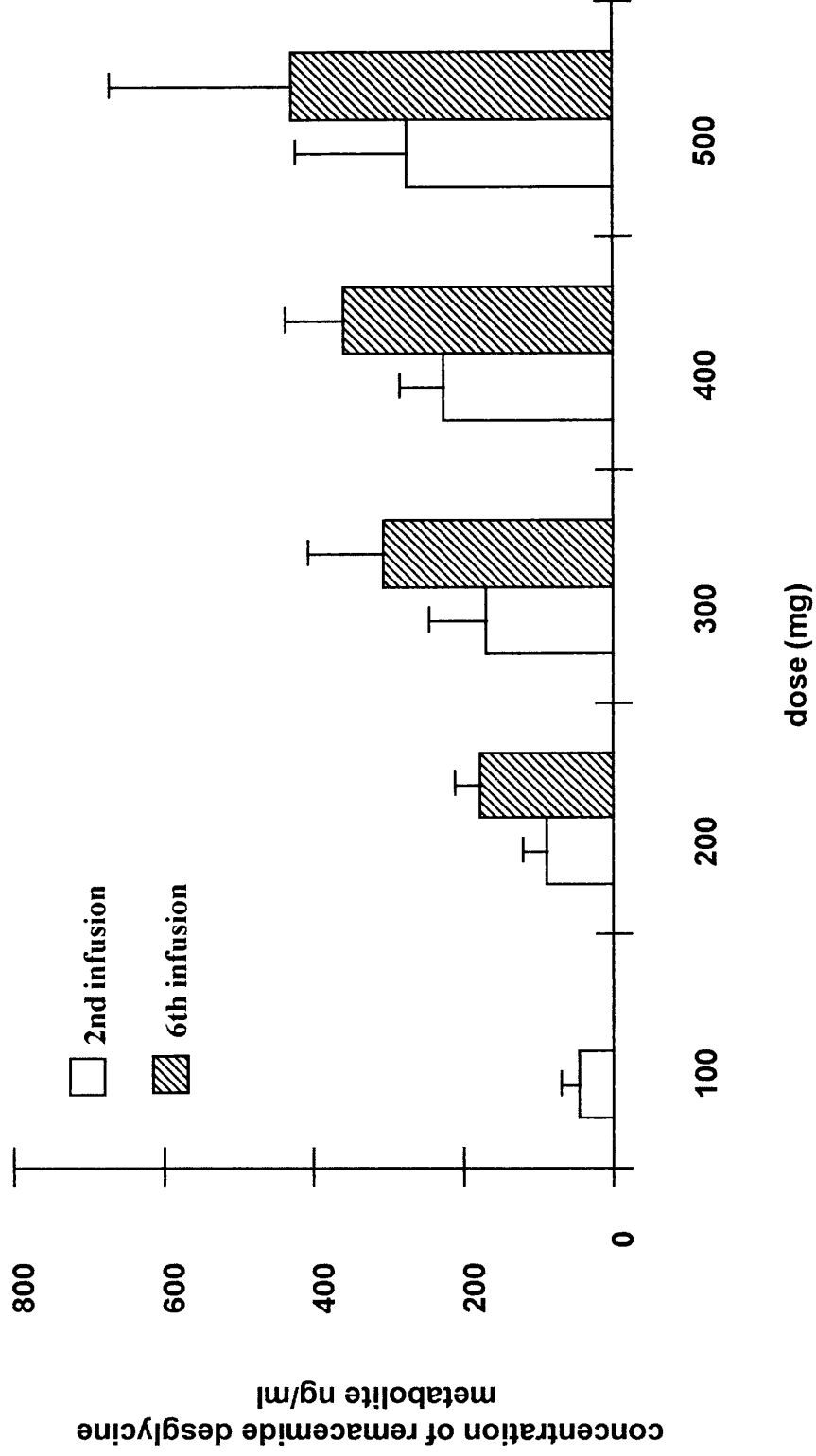


Figure 6.7.6.1: Functional outcome in patients receiving remacemide and placebo measured by Barthel score. There was no difference in functional outcome between patients receiving remacemide and placebo.

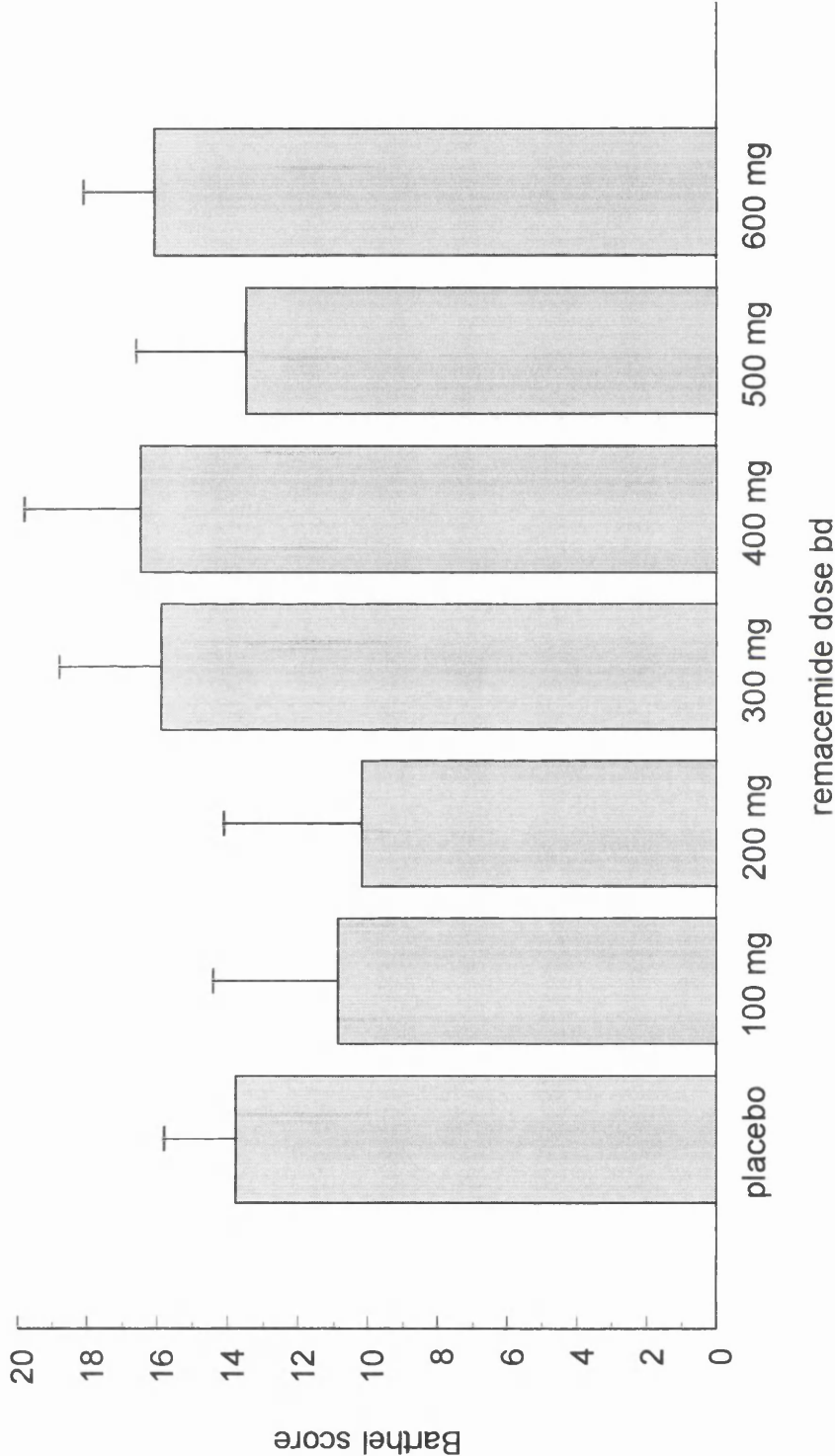
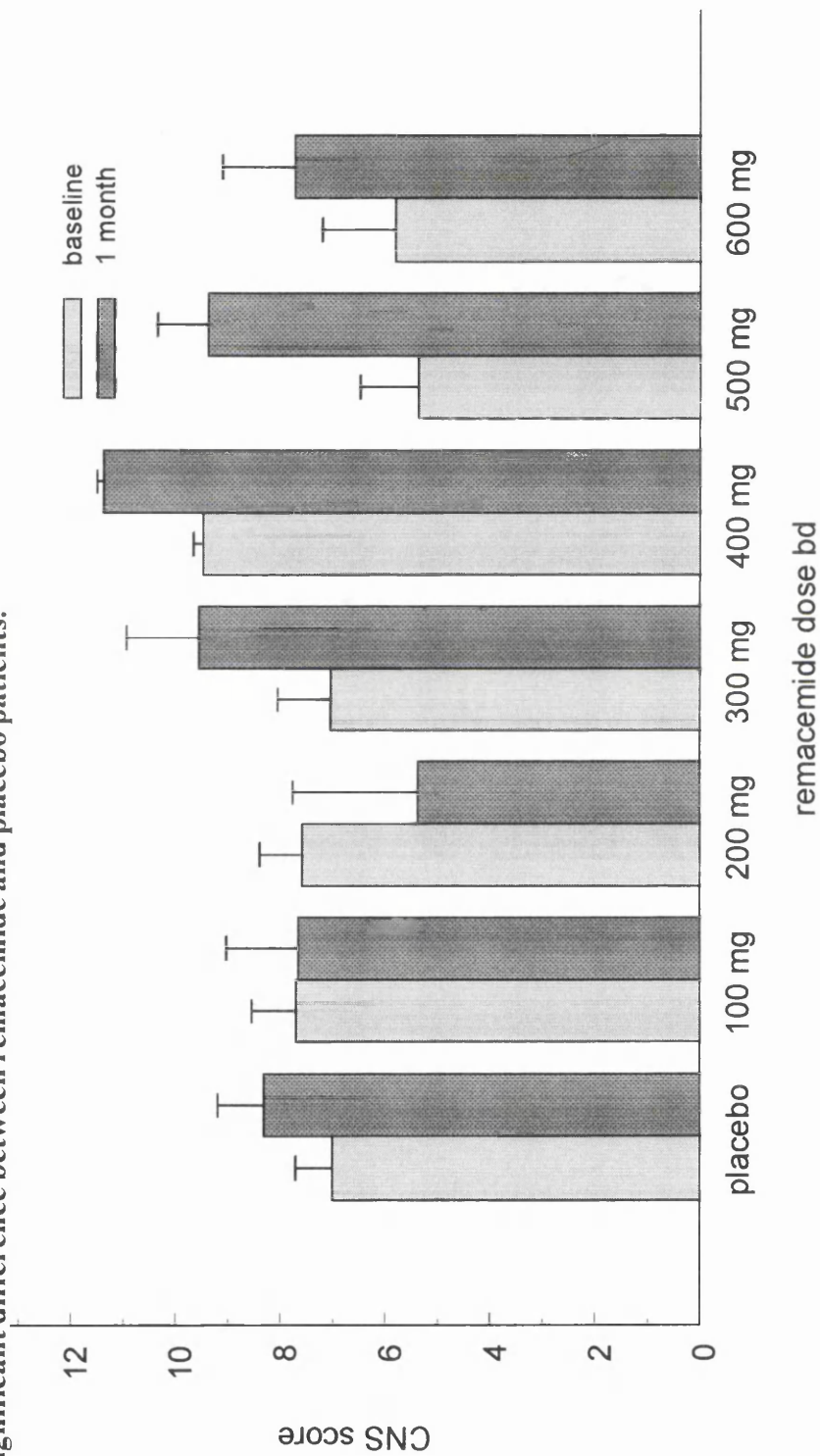


Figure 6.7.6.2: Neurological outcome expressed in terms of the Canadian Neurological Scale in patients receiving placebo or remacemide. There was no significant difference between remacemide and placebo patients.



Chapter 7

Chapter 7

A Double-Blind, Randomised, Placebo Controlled, Safety and Tolerability Study of Single Doses and Extended Infusions of Aptiganel HCl in Patients After an Acute Ischaemic Stroke.

7.1 Background

Neuronal damage after stroke is at least partly mediated by the build up of neurotoxic excitatory amino-acids. In physiological conditions glutamate acts as a neurotransmitter and its levels are regulated by reuptake and by conversion to glutamine in glial cells. Following ischaemic brain injury excess glutamate release coupled to a failure of these regulating processes leads to massive increase of glutamate concentrations. This activates the post-synaptic N-methyl-D-aspartate (NMDA) receptor, a ligand gated ion channel which in turn mediates influx of sodium and calcium leading to neurotoxicity. Cell culture of neurones with excess glutamate leads to loss of neurones that can be alleviated by antagonism of the NMDA receptor site. Similarly in animal models of stroke, administration of antagonists of the NMDA receptor can reduce the volume of cerebral infarction obtained after a standardised vascular insult by about 50%. Several NMDA antagonists are in preclinical and clinical development but as yet none are licensed for use outwith clinical trials. Aptiganel (CNS1102; proprietary name 'Cerestat') has been developed as a neuroprotective agent for the treatment of ischaemic and traumatic brain injury. In all subsequent references the compound will be named as aptiganel.

7.2 Preclinical pharmacology

Aptiganel [N-(1-naphthyl)-N-(3-ethyl phenyl)-N-methyl guanidine hydrochloride is a selective non-competitive antagonist within the ion channel pore of the NMDA receptor. In-vitro studies show it to be a high affinity antagonist that is neuroprotective in cultures of brain cells exposed to toxic concentrations of the excitatory amino acid glutamate.

Aptiganel reduced cerebral volume measured histologically and by diffusion-weighted MRI scanning after permanent MCA occlusion in rats. In this study reductions of 66% in infarct volume were reported suggesting aptiganel was a potent neuroprotective within this model. The putative neuroprotective dose was 3mg/kg and the minimal therapeutic concentration was 10ng/mL.²⁴⁹ Similar results were obtained in other permanent and reperfusion models of rat brain ischaemia. The neuroprotection achieved within these animal models was comparable with other neuroprotective compounds currently undergoing development.^{250,251}

Neurological effects similar to those seen with other NMDA antagonists in animals and man (ataxia and tremors) were observed after single intravenous infusion of 3mg/kg in rats and 2mg/kg/day in cynomolgus monkeys. Vacuolisation of the retrosplenium of the rat brain is induced by doses of aptiganel ≥ 10 mg/kg. These changes are associated with the appearance of behavioural disturbances in animals equivalent to the psychotomimetic effects seen in humans. Aptiganel is however 10 times less potent at inducing such changes than the NMDA antagonist MK-801. The clinical significance of these pathological changes is unknown but vacuolisation is reversible. There are no data available at present on the ability of these drugs to induce vacuoles in humans or sub-human primates. The anaesthetic agent ketamine, which has similar vacuole inducing properties has been used safely as an anaesthetic agent for many years with no long term sequelae.

Intravenous administration of aptiganel caused hypotension in anaesthetised rats but rises in blood pressure in conscious pigs and baboons. In animals, experimental interactions of agents active at the NMDA receptors with the regulation of the cardiovascular system are complex and dependent on the site of administration and the state of anaesthesia. Non-competitive NMDA receptor antagonists prevent glutamate stimulated blood pressure rises in the rat when administered intrathecally.²⁵² This is thought to be mediated by blockade of spinal NMDA receptors which mediate sympathetic vasoconstrictor outflow from the medulla. Direct injection of NMDA antagonists into the dorsal ventrolateral medulla is however associated with a rise in blood pressure.²⁵³

Cardiovascular effects of aptiganel in animals are therefore variable and are both species and conscious level specific.

7.3 Clinical experience

Aptiganel has been given as a 15 minute bolus to 21 healthy male volunteers within a double blind ascending dose study. Doses of up to 30µg/kg were well tolerated with only mild symptoms reported. Clinically significant sedation, raised mean arterial pressure and pulse rate were reported at doses > 30µg/kg. Symptoms progressed to disinhibition, nystagmus, diplopia and severe sedation at 45µg/kg. Doses of 60-100µg/kg were associated with paranoid ideation, hallucinations, peripheral vasoconstriction and catatonia (a state of severe sedation, unresponsiveness and immobility but preserved eye opening). Dose dependent rises in blood pressure were noted. Blood pressure began to rise within minutes of the infusion being commenced, peaked at 40 minutes after the end of the infusion, and resolved by 4 hours.¹⁸¹

Similar results were found when prolonged 4 hour infusions were given to male volunteers. Lightheadedness, dizziness, paraesthesia and euphoria were all reported at total doses of 32µg/kg. Doses higher than this (50 or 73µg/kg) were associated with intolerable side effects including motor retardation, perceptual disturbance and hallucinations. Giving aptiganel as a prolonged infusion therefore did not increase the total dose which could be safely administered to volunteers (~30µg/kg).

Pharmacokinetics of aptiganel obtained from volunteer data are summarised in the table below. Clearance was 125 ± 55 L/hr (mean ± SD) and the terminal t_{1/2} was 4.6 ± 2.9 h (mean ± SD).

The use of weight adjusted doses did not reduce the variability in the C_{max} and AUC. Pharmacokinetic analysis indicated little correlation between subject weight and volume of distribution (Vdss) [r² = 9.1%] or between weight and clearance (CL), suggesting dose adjustment for weight would not be necessary.

Summary of pharmacokinetics from bolus + 4 hour infusion study in healthy male volunteers

dose (µg/kg)	15	32	50	73
number of volunteers	3	6	3	3
Vdss(L)	639	500	596	452
C _{max} (ng/ml)	4.2	7.2	9.1	21.1
AUC(h.ng/ml)	6.3	20.4	48.7	52.5
Clearance(L/h)	198	125	82	97

The CNS symptoms and signs seen with aptiganel are similar to those seen with ketamine and phencyclidine. Symptoms closely resemble the dissociative anesthetic state reported with these drugs.²⁵⁴

Blood pressure effects of ketamine have been attributed to activation of catecholamines but in these volunteer studies there was no evidence of catecholamine excess or activation of the renin-angiotensin system.²⁵⁵ The hypertensive effect of aptiganel remains incompletely understood but is thought to be centrally mediated.

In a phase II clinical study in stroke patients tolerability was better than in volunteers with doses of up to 110 ug/kg tolerated without significant neurological and haemodynamic effects.²⁵⁶

Significant rises in blood pressure could potentially increase blood flow to an ischaemic area. While this may be of benefit in terms of reperfusing the penumbral zone it may conversely increase infarct size by exacerbating reperfusion injury and cerebral oedema. In theory increases in blood pressure could increase the risk of haemorrhage into infarction by the same mechanism. Total cerebral blood flow as measured by carotid Doppler was unaffected by the haemodynamic changes but middle cerebral artery velocity was increased. Peripheral vasoconstriction was seen in those volunteers exhibiting blood pressure rises.²⁵⁵

7.4 Aims and objectives

Primary

Results from experimental animal models of stroke suggest plasma concentrations > 10 ng/ml are required for neuroprotection. The purpose of the study was to determine the maximally tolerated non-weight adjusted bolus dose of aptiganel and to define an appropriate infusion to maintain plasma levels at steady state.

Secondary

The study was not powered to demonstrate efficacy but preliminary data were collected. The use of weight-independent doses was also being explored to confirm the independence of clinical effects on patient weight.

7.5 Patients and methods

7.5.1 Study design

This was a multi-centre, double-blind, randomised, placebo-controlled safety and tolerability study of ascending doses of aptiganel in two phases. The first phase (part A) consisted of single ascending doses and the second (part B) a bolus dose followed by an extended infusion.

Ethics and consent

Ethical approval was gained from the local West Ethics committee. Patients gave written, witnessed informed consent whenever possible. Where patients were unable to write, witnessed verbal consent was accepted from the patient. All witnesses were independent and

had no connection with the study. If the patient was unable to give consent verbally written informed assent was accepted from the next of kin or other close family member.

7.5.2 Patient selection

Inclusion criteria

All patients with acute neurological deficit consistent with a diagnosis of carotid artery territory stroke were considered eligible for the study, providing they met the criteria described below.

- Acute neurological deficit of at least one hour's duration but ≤ 24 hours (for night-time stroke time of onset taken as last time patient was known to be free of stroke symptoms)
- Males or non-fertile females (post-menopausal or surgically sterilised)
- Requiring hospitalisation
- 21 years of age or older
- Weight 50-150 kgs inclusive
- Minimum of 4 and maximum of 20 on the baseline NIH scale
- Symptoms consistent with carotid artery territory stroke
- Witnessed informed consent or consent from an authorised representative.
- CT scan consistent with diagnosis of ischaemic stroke
(scan was performed prior to study entry)

Exclusion criteria

Patients with any of the following were excluded from the study:

- Coma or stupor (unable to localise pain)
- Evidence of brain tumour, cerebral oedema or haemorrhage on initial CT scan prior to entry
- Malignant hypertension defined as bilateral fundal haemorrhages and/or papilloedema + hypertension i.e. blood pressure > 200/110 on at least 2 separate readings
- Significant hypotension i.e. blood pressure below 90/50 mm Hg
- Any other unstable medical condition which would interfere with the assessment of study treatment
- Involvement in any other investigational drug study within 3 months

7.5.3 Dosing regimen

In the first phase of the study patients received drug or placebo diluted to 10 mls with normal saline. This was administered over 3 to 5 minutes by bolus intravenous injection. Bolus doses were as follows: **3mg, 4.5mg, 6mg and 7.5mg**. Patients were randomised to receive active drug or placebo in a ratio of 3:1 active/placebo for part A. Randomisation was by a telephone accessed central computerised system.

In part B two well tolerated bolus doses were chosen from the first phase and followed by a continuous infusion. Patients were randomised in a ratio of 4:1 active/placebo. The constant infusion was administered for 6 hours initially with an option to continue to 12 hours if well tolerated. Rates of infusion in part B were based on pharmacokinetic data already available

from part A and previous studies. The first group of patients received a 6mg bolus dose followed by 1mg/hour. Further assessment at this dose was abandoned due to the high frequency of intolerable side effects. A lower dose was then administered and these patients received a 4.5mg bolus followed by 0.75mg/hour. Both continuous infusion doses were predicted to achieve and maintain plasma aptiganel levels above the projected neuroprotective concentration of 10 ng/ml.

7.6 Study procedures

7.6.1 Screening

Patients were screened at the time of admission. This assessment ensured that all inclusion and exclusion criteria were met. Baseline clinical haematology, biochemistry and ECG assessments were carried out. The patients were examined by a member of the medical staff who documented the neurological deficit and ensured the diagnosis was consistent with an acute stroke.

7.6.2 Safety

Routine biochemistry, haematology, and urinalysis were carried out by standard laboratory methods. Blood and urine were taken for analysis at presentation, 24 hours following start of drug administration and at 7 ± 2 days. Serious adverse events occurring throughout the study period were continuously documented as they occurred.

Biochemical, and haematological parameters measured are documented in the table below.

Biochemistry	haematology	urinalysis
sodium	total white blood cells	blood
potassium	red cells	protein
glucose	haematocrit	ketones
urea	haemoglobin	glucose
creatinine	platelet count	specific gravity
calcium	prothrombin time	
bilirubin	lymphocytes	
albumin	monocytes	
ALT	eosinophils	
AST		
LDH		
alkaline phosphatase		

7.6.3 Tolerability

Adverse events were continuously documented as they occurred. An assessment was made of the treatment’s acceptability 24 hours after each administration by the individual investigators involved. This took into consideration the overall impression of the symptomatic and haemodynamic side effect profile.

7.6.4 Vital signs

Patients were monitored by repeated physical examinations and recording of vital signs.

Lead II ECG and vital signs were recorded at the following times.

- 0 (bolus injection time)
- 5, 10, 15, 20, 25, and 30 minutes from bolus injection time.
- 40, 50 and 60 minutes from bolus injection time.
- 2, 2.5, 3, 3.5, 4 hours from the bolus injection time.

In part B additional recordings were made at:

- 5, 6, 7, 8, 9, 10, 11 and 12 hours from bolus injection time and at 1, 2, 3, 4 hours after the end of the infusion.

7.6.5 Pharmacokinetics

Pharmacokinetic (PK) data were collected during part A to allow prediction of appropriate doses for part B. Samples were drawn from an indwelling catheter sited in the contralateral arm from that being used to infuse drug at:

- 30 minutes, 2, 6 and 12 hours from the bolus infusion time.

In part B, PK samples were taken at:

- 30 minutes
- 2, 6, 12, 24 hours from the start of the infusion.

Plasma was obtained by collecting supernatant following centrifugation at 3000 revs/ minute for 10 minutes after which samples were frozen and stored at -20 ° C.

Plasma drug concentrations were determined by HPLC with UV detection, validated in the range 1.25 - 100 ng/ml. Intra-assay coefficients of variation were ranged from 1.0 to 2.5%. Inter-assay coefficients of variation were under 4%.

7.6.6 Outcome

The study was not powered to demonstrate efficacy but neurological outcome was measured using NIH (appendix I). Functional outcome was assessed by the Barthel Index (appendix III). NIH Stroke Scale assessments were made at screening, 24 hours after bolus dosing and at 7 ± 2 days. Functional Barthel index was carried out on day 7 ± 2 .

7.7 Results

7.7.1 Recruitment and demographics

Four clinical sites participated in the study: three in the US and one in the United Kingdom. Forty six patients were admitted to the study: 36 received aptiganel and 10 were given placebo.

Part A

Four dose groups were evaluated in an arithmetic progression i.e. 3mg, 4.5mg, 6mg and 7.5mg. A total of 21 patients were recruited to this initial part of the study: 16 received aptiganel and 5 placebo. At least four patients were evaluated in each dose group prior to dose escalation. Demographic details of patients entered into Part A are summarised in the table below.

Demographics of patients admitted to part A of the study.

Bolus administration only.

	Placebo	3.0 mg	4.5 mg	6.0 mg	7.5 mg	Total receiving drug
number of patients	5	3	3	4	6	16
male	2	2	1	3	1	7
female	3	1	2	1	5	9
weight (kg)						
mean	69	66	71	94	80	79
range	51-91	51-84	57-99	84-113	64-124	51-124
Age (years)						
mean	78	69	59	74	67	67
range	71-84	47-81	28-83	69-76	47-77	28-83

Part B

The dose initially considered to be the most appropriate for further study was a 6mg bolus followed by an infusion of 1mg/hour.

Twenty-five patients were recruited to part B of the study: 20 received drug and 5 placebo. Eight received a 6mg bolus followed by an infusion of 1mg/ hour for a maximum of 12 hours. Due to side effects at this dose recruitment was discontinued and a new lower dose assessed i.e. 4.5mg followed by 0.75mg/hour. A total of 12 actively treated patients were evaluated at this lower dose. Demographic details from part B are summarised in the table below.

Demographics of patients admitted to part B of the study. Bolus plus infusion.

	Placebo	6.0+1.0mh/hr	4.5+0.75mg/hr	total receiving drug
number of patients	5	8	12	20
male	1	2	6	8
female	4	6	6	12
weight (kg)				
mean	95	76	72	73
range	59-118	50-119	43-126	43-86
age (years)				
mean	77	69	71	70
range	67-86	43-85	44-86	43-86

7.7.2 Compliance

Compliance with the protocol was satisfactory. Four patients were discontinued from the trial following the serious adverse events documented below. Two patients had the infusion discontinued for a short period to allow resiting of iv access but in both cases the correct volume of infusate was given by the end of the study.

All appropriate clinical and Stroke Scale assessments were carried out at the appropriate time. Biochemistry and haematology at discharge were omitted in patient 301 (6+1mg dose).

7.7.3 Part A Serious adverse events

Mortality

There were three deaths in part A of the study. No deaths were thought likely to be attributable to study treatment.

Placebo

One placebo patient died from a recurrent stroke on day 21 after treatment.

Aptiganel

The other two deaths received active treatment at the 6mg dose and both died 3 days after treatment. One 78 year old patient suffered a stroke following pacemaker insertion. He was known to have complicating congestive cardiac failure, pulmonary and systemic hypertension and coronary heart disease. He died of respiratory failure following an episode of pneumonia. A 75 year old male with a history of coronary heart disease died as a result of a respiratory arrest following a large hemispheric cerebral infarction.

Part B

There were no deaths during either the study or the follow up period.

Part A Serious Non fatal events

CNS effects

CNS effects were reported equally frequently throughout the placebo, 3, 4.5 and 6mg dose groups. CNS effects were both more frequent and severe in the 7.5mg dose group (tables 7.7.3.1 and 7.7.3.2). Six patients were treated at this dose. Catatonia or marked eyes open sedation previously reported in volunteer studies at a dose of 100 mcg/kg was reported in one patient and hallucinations in another. Three patients became significantly confused. Severe sedation and hallucinations in addition to BP effects discussed below precluded further evaluation of this dose and thus the lower bolus dose of 6mgs was chosen for initial assessment in part B.

Haemodynamic effects

Rises in systolic blood pressure were noted in several patients receiving active treatment compared with a BP fall in those receiving placebo. Rises in systolic blood pressure became increasingly frequent at the higher doses. One patient (102) out of 3 patients at the 3mg dose had a significant rise in blood pressure (>35 mmHg). Blood pressure, in this individual, rose from 122/76 mmHg to a peak of 198/88 one hour after dosing (figure 7.7.3.1). One of 3 patients at the 4.5mg dose (203) exhibited a less dramatic increase in BP from 148/73 at baseline to 202/95 at 2.5 hours (figure 7.7.3.2). Two of three patients at the 6mg dose had rises of 44 (302) and 38mmHg (303) respectively (figure 7.7.3.3). In the highest dose group (7.5mg) 3/6 actively treated patients suffered significant rises in BP. Patient 404 had a rise from 133/74 baseline to 182/76 (49 mmHg rise in systolic blood pressure); 407 rose from 164/83 to 224/114 (60 mmHg); 403 from 154/74 to 216/80 (62 mmHg). Patient 403 was given a single sub-lingual dose of nifedipine to reduce blood pressure 40 minutes after the bolus injection. The remaining three patients' blood pressure varied consistent with expected fluctuations (figure 7.7.3.4). The average rise in BP within the 7.5mg dose group (34 mmHg)

was considered too high for further evaluation in part B (figure 7.7.3.5). There were no significant rises in blood pressure within the placebo group.

Part B Withdrawals

During infusion

In total 4 patients had their medication discontinued. Three received the 6mg bolus/ 1mg per hour infusion and 1/8 was withdrawn from the lower 4.5mg bolus followed by 0.75mg/ hour group. The AE details and times of stopping study drug are described in the table below.

Patient number	dose	duration of infusion	reason for discontinuing drug
903 HS	6mg+1mg/hr	11 hours	Patient became rigid and unresponsive
908 KSB	6mg+1mg/hr	43 minutes	Patient developed marked subendocardial ischaemia associated with 30mmHg rise in mean blood pressure
910 BT	6mg+1mg/hr	60 minutes	50mmHg rise in blood pressure with numerous PVB's on ECG monitoring
961 RDD	4.5mg+0.75mg/hr	4 hours 15 minutes	Patient respiratory function deteriorated: cyanosis, reduced respirations and oxygen saturation

CNS effects

CNS and psychotomimetic effects were severe and frequent at the 6mg/1mg per hour dose (tables 7.7.3.1 and 7.7.7.2). Six out of eight patients in this group developed profound sedation. Five patients developed symptoms within 2 hours of administration and 4 of these 5 required discontinuation of study drug infusion. One patient in the high dose group had late onset of sedation 11 hours following administration.

Further recruitment to this group was terminated and a lower dose selected for further study. Only one patient in the 4.5mg / 0.75mg group developed significant sedation but symptoms were severe and prolonged lasting 4 days (patient 958). In this individual sedation was also associated with agitation and hallucinations. It is unclear whether this duration of symptoms was due to a drug associated event, or a neurological deterioration related to the underlying stroke.

Vital signs

In the group receiving 6mg+1mg/ hr three out of eight patients were treated for hypertension due to blood pressure increases resulting in systolic BP of > 210 mm Hg. Patient 909 is one such patient who required iv labetalol for control of blood pressure. The BP profile is summarised in figure 7.7.3.6 as an example. There was a significant rise in the mean arterial blood pressure in this group of patients (figure 7.7.3.7).

Within the 4.5mg + 0.75mg/hr dose group there was an average 30 mm Hg rise in systolic blood pressure. Four out of eight of the patients recruited to this lower dose infusion group required anti-hypertensive treatment. Patients within this group who received anti-hypertensive therapy had dramatic reductions in blood pressure following treatment. Figure 7.7.3.8 gives an example of the blood pressure profile in a patient requiring sub-lingual

nifedipine for control of blood pressure and figure 7.7.3.9 summarises the mean blood pressure changes over time.

Other adverse events

Systemic adverse events were no more frequent in the treated patients compared with placebo.

Gastrointestinal upsets were more common in the lower 4.5mg + 0.75mg dose group compared with placebo or 6+1mgs. 5 patients complained of dyspepsia while 3/12 had episodes of vomiting. Urinary complaints were more commonly reported as adverse events in patients receiving extended infusions. This was associated with the insertion of Foley catheters leading to discomfort, irritation, infection and abnormal urinalysis results.

7.7.4 Laboratory results

Analysis of haematology, urinalysis and biochemistry did not reveal an excess of abnormalities in the aptiganel receiving patients. Urinalysis were positive for blood and protein in those patients who required catheterisation but there was no excess in those receiving drug rather than inactive placebo. No significant excess of ECG abnormalities were reported in the actively treated patients.

7.7.5 Treatment acceptability

All treatment allocations were determined to be either acceptable or unacceptable based on the safety and tolerability profile for each individual. This assessment was made by the investigator personally responsible for the management of that individual patient and took into consideration the patient's own perception of acceptability as well as the physician's.

Part A

All placebo receiving patients treatments were considered acceptable as were all patients receiving active treatment with bolus doses of 3, 4.5, and 6mg. 2/6 individual treatments at the 7.5mg bolus dose were considered unacceptable. Patient 406 found psychotomimetic effects unacceptable as did patient 407 who also experienced an asymptomatic rise in blood pressure of > 30/15 mmHg which was of concern to the treating physician.

Part B

Four out of seven treatments in the 6 + 1mg group were deemed to have been unacceptable by the investigating physicians. Patient 904 developed a hypertensive crisis, generalised catatonia and hallucinations. Patient 903 developed catatonia and rigidity 11 hours after the maintenance infusion had been commenced. Patient 908 developed generalised subendocardial myocardial ischaemia and the maintenance infusion was stopped after 43 minutes. This was associated with a significant rise in systolic blood pressure. Patient 910 developed catatonia and multiple ectopic beats on cardiac monitoring.

Of those treated at the 4.5 + 0.7mg dose; patient 953 developed significant sedation and 954 developed nausea and vomiting. Both effects were considered to be acceptable with

reservation expressed by the investigators concerned. It is possible that the nausea and vomiting were not drug effects as these symptoms are frequently reported in stroke patients including the placebo receiving patients in the control group.

7.7.6 Pharmacokinetics

Part A

Mean concentrations of aptiganel for the bolus doses are shown in figures 7.7.6.1, 7.7.6.2, 7.7.6.3 and 7.7.6.4. Patient weight did not correlate with plasma concentrations achieved. Concentrations above the putative neuroprotective drug concentration were obtained in patients receiving the 4.5mg doses and above.

Part B

Plasma levels achieved during the two prolonged infusion regimens did not differ significantly from each other ($p = 0.35$ ANOVA). Concentrations achieved by both doses exceeded putative neuroprotective levels (figure 7.7.6.5). Infusions were, however, prematurely discontinued in the 6mg + 1mg infusion group in 3 / 8 patients. Plasma pharmacokinetics remained linear and clearance was unaffected by the duration of infusion. Patient weight did not influence concentrations achieved. There was no correlation between plasma levels achieved and the severity of side effects reported. The C_{max} ranged widely, from 10-21 ng/ml, in those patients whose side effects required discontinuation of infusion. Pharmacokinetics are summarised in the table below.

Summary of clinical pharmacokinetics

Dose group mg	Cmax ng/mL	Clearance mL/min	Vdss L	n
3	5.8	1565	371	3
sem	(0.6)	(536)	(53)	
4.5	11.1	1557	367	2
sem	(1.8)	(101)	(34)	
6	13.5	1557	480	4
sem	(3.4)	(493)	(112)	
7.5	16.3	1532	365	6
sem	(1.5)	(137)	(31)	
6+1	16.5	1228	540	8
sem	(1.4)	(129)	(57)	
4.5+0.7	14	1224	547	11
sem	(2.1)	(208)	(91)	

7.7.7 Clinical outcome

The NIH stroke scale was assessed at admission and then at discharge. Most recordings were obtained between 5 and 7 days post treatment, but the variability in time to discharge was wide. The power of the study was not sufficient to test for drug efficacy. There was no significant difference between placebo and actively treated groups in terms of the NIH Stroke Scale or Barthel index (figures 7.7.7.1 and 7.7.7.2).

7.8 Discussion

In a population of stroke patients a non-weighted adjusted dose of 4.5mg followed by an infusion of 0.75mg/hour achieves and maintains plasma levels thought to be neuroprotective in animals (10 ng/ml). This dosing schedule appears reasonably well tolerated, but is associated with notable haemodynamic effects (a mean rise in systolic blood pressure of 30mmHg). Sedation was reported in 1/12 individuals but the symptoms were both prolonged and severe. Whether or not the reported sedation was a true drug effect will only be answered by a larger study.

Higher dose infusions of 6mg + 1mg per hour infusions were associated with more dramatic increases in blood pressure and frequent reporting of intolerable CNS side effects of agitation and catatonia. The assessment of this dose was abandoned as the haemodynamic and side effect profile was unacceptable to both investigators and patients.

It is always possible that a study with an ascending dose design will be prematurely discontinued due to clustering of chance adverse events. Similarly the acceptable tolerability obtained at a lower dose may not be repeated in a larger study. This potential bias can be addressed by treating larger numbers of patients at the doses of interest. In this particular study the side effects and adverse events reported at the 6mg+1mg/h dose were so dramatic and consistent with experience from CNS-1102 volunteer and other NMDA antagonist studies as to suggest further evaluation of the 6mg + 1mg/ hr dose to be unwise, if not unethical. It remains conceivable that a larger phase III trial could report a higher frequency of adverse events at the 4.5+0.75mg /hr dose.

Most patients within the 6mg + 1mg/hr dose group had increases in blood pressure. Blood pressure was also increased to a lesser extent within the 4.5mg + 7.5mg group and settled to levels comparable with placebo after 4 hours.

Haemodynamic changes are likely to influence cerebral haemodynamics which could positively or negatively influence stroke outcome. At the time of cerebral ischaemia, cerebral autoregulation is lost. Thus changes in blood pressure could directly influence changes in local cerebral perfusion particularly in penumbral areas. Reductions in blood pressure, i.e. those induced by treatment that may be given to counteract the effects of aptiganel could potentially reduce the local cerebral perfusion of the ischaemic area. While moderate rises in blood pressure may be beneficial by increasing blood flow to ischaemic areas of brain dramatic increases in blood pressure such as those obtained with higher doses of aptiganel could potentially lead to an increased risk of reperfusion injury and even haemorrhagic transformation of infarction.

While studies of aptiganel in human volunteers suggest that as blood pressure is increased there is no change in total cerebral blood flow, this may not be the case in stroke patients. In addition total cerebral blood flow may be unaltered while local perfusion to ischaemic areas changes significantly.

Treatment which lowers blood pressure in the acute stroke phase has been reliably demonstrated to worsen clinical outcome. This was reported in the INWEST trial of intravenous and oral nimodipine therapy in patients with acute stroke⁵⁴ and also in a study of the neuroprotective ion channel blocker lifarizine.⁵⁵ As yet the clinical effects of a moderate increase in blood pressure similar to that observed in the lower infusion dose group is undefined.

In conclusion the 4.5mg/0.75mg/hour dosing regimen was not associated with excess CNS or psychotomimetic effects when compared with placebo, but was associated with a hypertensive effect. Further evaluation of the efficacy of the 4.5mg + 0.75mg/hour schedule is merited within a Phase III clinical efficacy study, but haemodynamic effects should continue to be carefully addressed.

Final conclusions and future prospects

The prevention and treatment of cerebrovascular disease remains a major challenge for physicians and researchers. While antihypertensive therapy is established as an effective form of primary prevention, studies continue to investigate its effectiveness in secondary prevention. Controversy exists regarding when anti-hypertensive therapy should be instituted. Our study indicates that commencing ACE inhibition between 3 and 7 days following stroke is both effective and safe.

There were two limitations of that study. First, patients with severe carotid disease were excluded on safety grounds that were valid at the time the study was designed. Knowing that the treatment was safe in patients with normal carotid arteries now allows us to consider extending treatment to patients with stenosis. Second, we confirmed that total carotid flow was unaltered by treatment but have not excluded effects on local perfusion in the peri-infarct region, as Doppler would be insensitive to flow redistribution. A further study has now been designed and ethics approval gained, that will deal with these points. It will also consider a third, previously disregarded, issue: it will investigate the incidence of clinically important renovascular disease in patients with cerebrovascular disease who are to be treated with an ACE inhibitor.

Patients with recent cerebrovascular disease will be recruited. They will be stratified according to presence or absence of moderate to severe occlusive carotid disease. After baseline screening of cerebral blood flow by both Doppler and SPECT scanning and after assessment of renal function by radio-isotope methods, patients will be randomised to

receive perindopril or placebo once daily for two weeks. The effect of perindopril on renal function, on total cerebral blood flow and on local perfusion will each be assessed.

The PROGRESS secondary stroke prevention study continues to study patients who may have occult carotid or renal artery stenosis. The results from the above proposed trial may assist in interpretation of the results of PROGRESS, and assuming that blood pressure lowering is proven beneficial, may guide selection, investigation or monitoring of patients in future years.

The work described in chapter 3 arose from a more general examination of risk factors in our patient population, and from testing of a hypothesis that patients with raised cholesterol had a greater risk of secondary events and should be offered cholesterol-lowering treatment. Our counter-intuitive results have certainly stimulated debate within the medical literature.²⁵⁷⁻²⁶¹ More important, most correspondents agree that a trial of cholesterol-lowering in elderly patients with cerebrovascular disease is not only ethical but essential. Debate continues over the entry criteria for such a study: should it be restricted to patients without evidence of ischaemic heart disease, should there be fixed age limits, should there be baseline cholesterol limits, which drug should be used to lower the cholesterol? We plan to resolve these issues in discussion with colleagues throughout the UK and to initiate a multicentre study within the next 12-18 months.

Chapter 4 has considered the role of hyperglycaemia in determining outcome after acute stroke. No single study can resolve the issue of whether the hyperglycaemia is truly independent of stroke or is a stress response. The study that we have published has, however, stimulated further debate and has prompted ourselves and others finally to initiate pilot trials of intervention for hyperglycaemia complicating stroke. Thus, we are randomising patients who present with a blood sugar of > 8mmol/L to receive standard medical care or tight control of blood sugar with insulin (target range 5 - 8 mmol/L). When possible, patients

also have a baseline MRI scan with diffusion- and perfusion-weighted imaging, followed after 1 month by a T₂-weighted MR scan. If insulin improves outcome, the imaging may assist in deciding whether it acts via a local effect to restrict infarct size or a systemic effect. Clearly, a multicentre study is likely to be required, and we have started to seek grant funding to collaborate on this with other European centres.

In chapter 5, I have described the initial administration to stroke patients of a novel glycine antagonist, GV150526. The results of that study have provided the essential information on which a choice of loading infusion and selection of maintenance doses have been based for two large phase 3 trials with this drug. The phase 2 study that I have undertaken not only demonstrated the good tolerability of GV150526 in comparison with other means of manipulating the NMDA receptor, but also confirmed the pharmacokinetics of GV150526 in stroke patients. When coupled with the new information on an effect of GV150426 on bilirubin transport/metabolism and/or hepatic function, it is now clear what the dose-limiting effects of this drug are in man, and clear that it is possible to administer sufficient drug to maintain putative neuroprotective plasma concentrations of GV50526 for several days after acute stroke. The fact that a single centre can establish so much information in under a year is impressive, and offers a lesson to other pharmaceutical companies. Only the phase 3 trials will answer whether outcome can be improved.

The lessons from remacemide, in chapter 6, have also been of considerable importance for future drug development. This was also a single centre study. We showed early on, that oral drug administration is impractical after acute stroke. We have established a maximum tolerated dose of remacemide that could be considered for future stroke trials. The study also suggested strongly that continued development of remacemide for this indication may be unwise: the adverse effects of the drug appear to be related to the active metabolite. The neuroprotective effects are probably also related to the metabolite. Since, with the administration regime that was used, the metabolite concentrations and the adverse effects

accumulated during repeated daily dosing, it is likely that neuroprotective concentrations were not attained until many hours after treatment was initiated. The use of a pro-drug such as remacemide would therefore delay effective neuroprotection and render it ineffective. The sponsoring company, Astra, have recognised this shortcoming and have since switched their interest to a related compound that has inherent activity, ARL15896AR. Studies with ARL15896AR have been conducted in our unit over the last year.

Finally, in chapter 7, another potential neuroprotective drug, aptiganel hydrochloride, was assessed. This study was crucial in confirming that weight-independent dosing was practical and safe, and in identifying the maximum tolerated loading and maintenance doses for future phase 3 stroke trials. Since then, a phase 3 stroke trial has been underway. At present, recruitment is on hold pending and interim safety and efficacy analysis.

Table 7.7.3.1: CNS Adverse events in patients receiving bolus doses of aptiganel. All CNS adverse events reported within 7 days of drug treatment. Number of specific events reported in each dose group.

Adverse event	placebo	3.0 mg	4.5 mg	6.0 mg	7.5 mg
	n=5	n=3	n=3	n=4	n=6
headache	1	1	1	0	4
visual disturbance	0	0	1	1	2
sedation	1	1	1	1	4
anxiety	2	1	2	1	2
confusion	2	2	2	1	3
hallucinations	0	0	0	0	2
paranoia	0	0	0	0	1
nystagmus	0	0	0	0	2
anarthria	0	0	0	0	1
lightheadedness	1	1	2	0	3
total	7	6	9	4	24
AE's per patient	1.4	2	3	1	4

Table 7.7.3.2: CNS Adverse events at respective bolus doses of aptiganel. Severity graded by patient: + mild, ++ moderate, +++ severe.

Dose	number	Symptom	confusion	depersonalisation	paraesthesia	sedation	visual disturbance	dizziness	agitation	hallucinations	catatonia	paranoia
3 mg												
	101					+						
	102					+		+				
	104		none	reported								
4.5 mg	202				+							
	203		+		+			+	+			
	204								++			
6 mg	301		none	reported								
	302				+	+	+					
	303		none	reported								
	305		none	reported								
7.5 mg	401		++	++								
	403			+		+						
	404			+			+	+				
	405					++						
	406					+++	+		++	++		

Figure 7.7.3.1: Blood pressure changes in patients receiving bolus doses of aptiganel; 3 mg dose. Patient 102

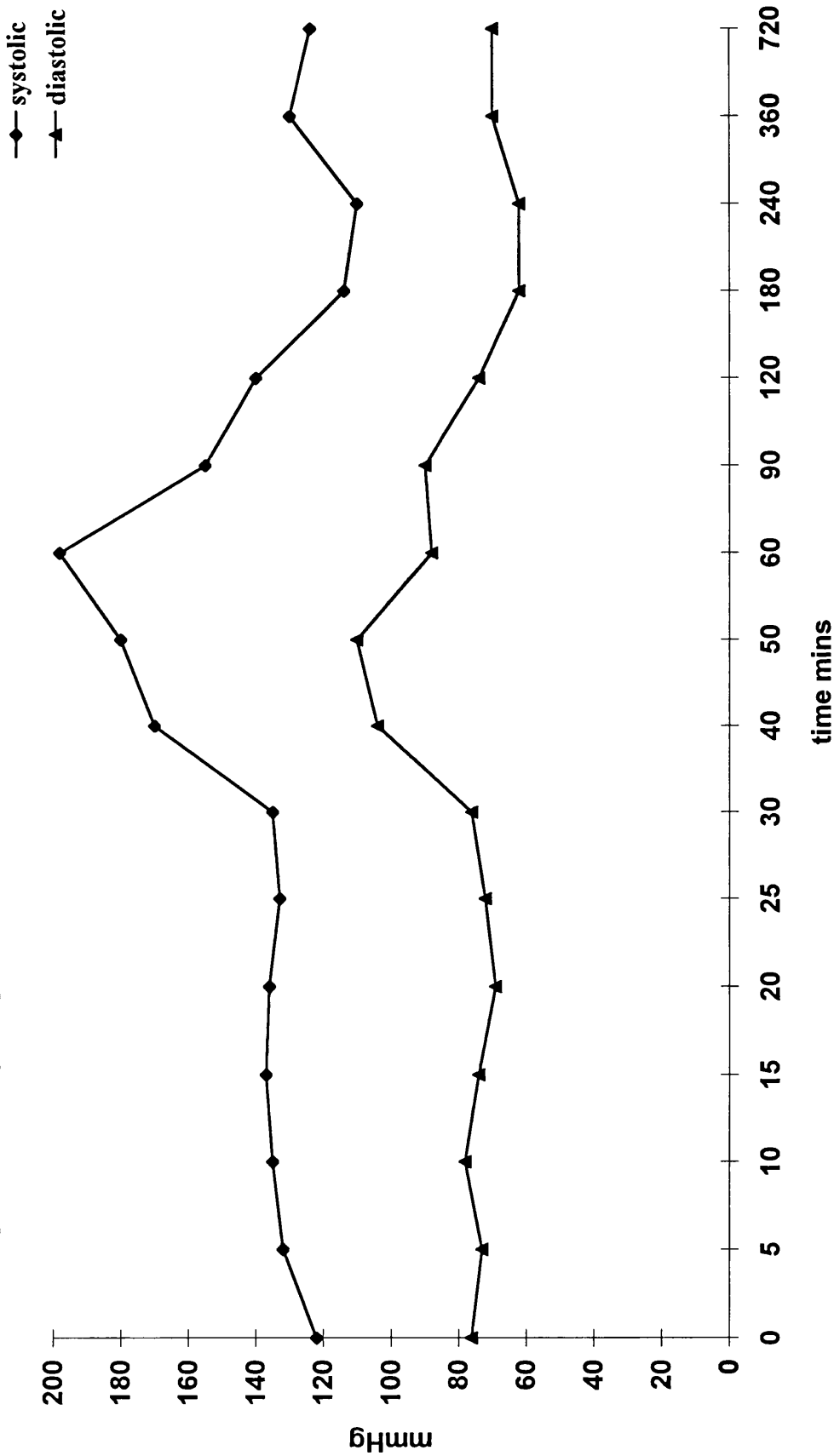


Figure 7.7.3.2: Blood pressure changes in patients receiving bolus doses of aptiganel: 4.5 mg dose. Patient 203

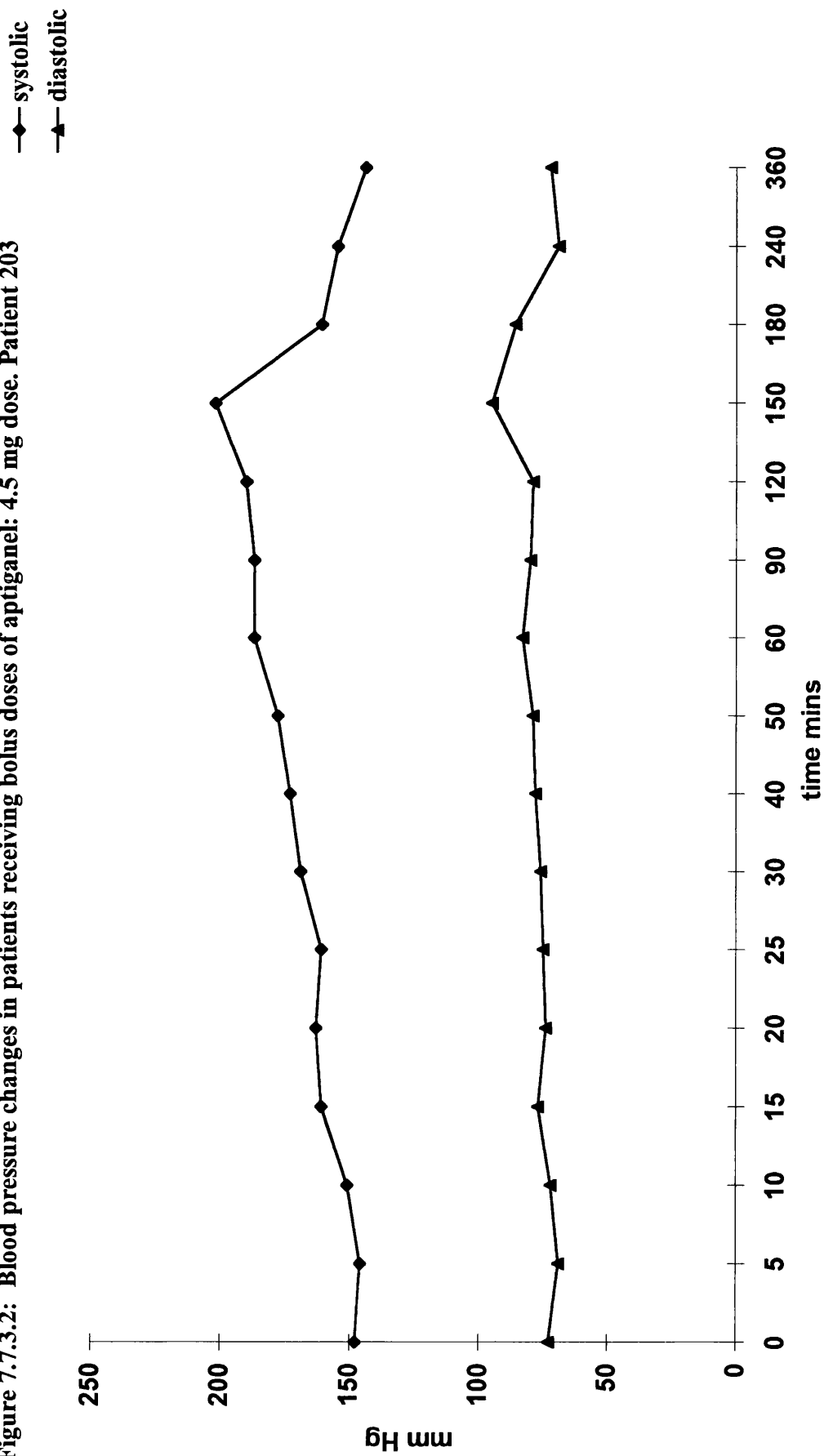


Figure 7.7.3.3: Blood pressure changes in patients receiving aptiganel 6 mg bolus

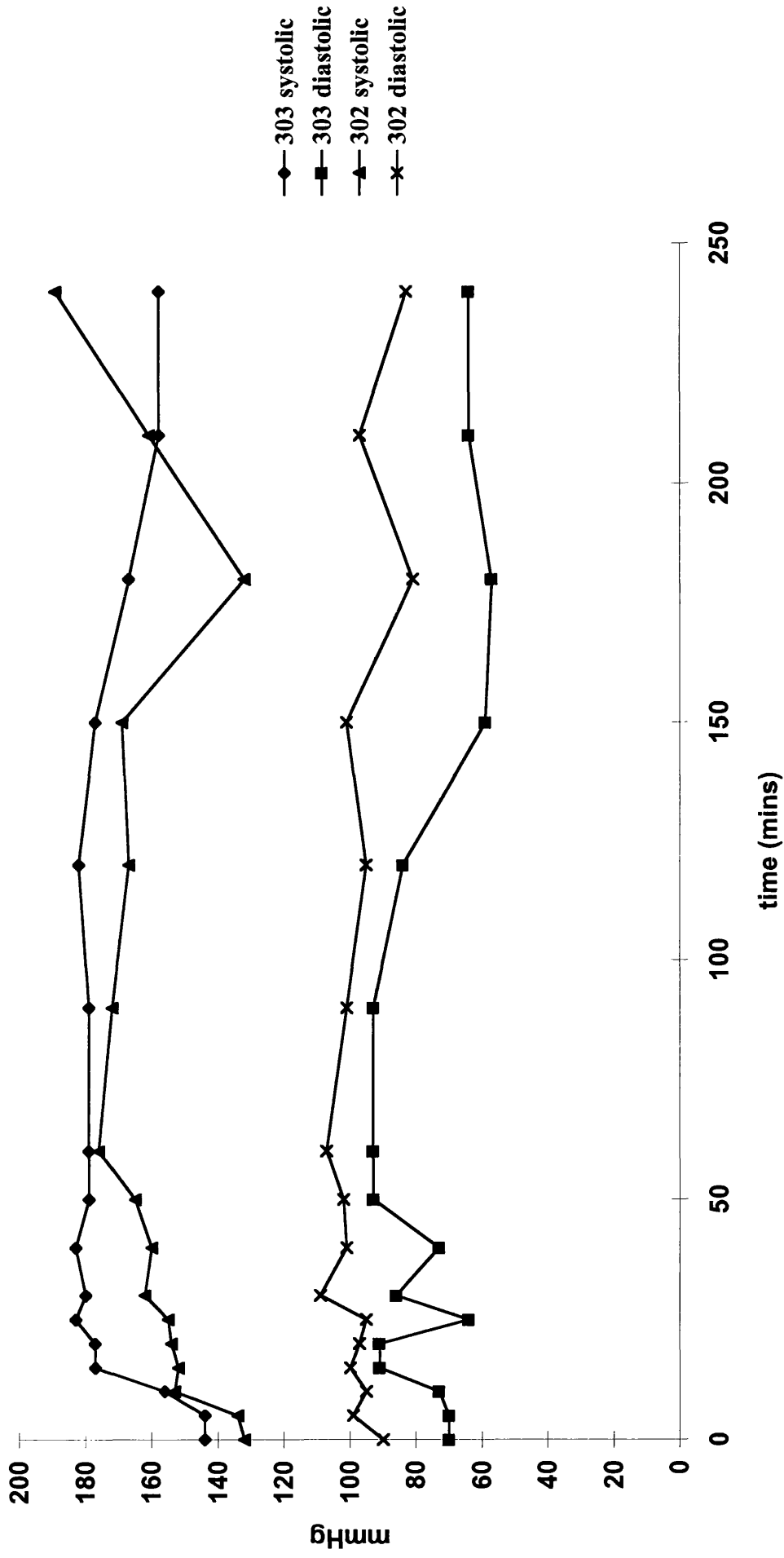


Figure 7.7.3.4: Blood pressure vs time in individual patients receiving aptiganel 7.5 mg bolus.

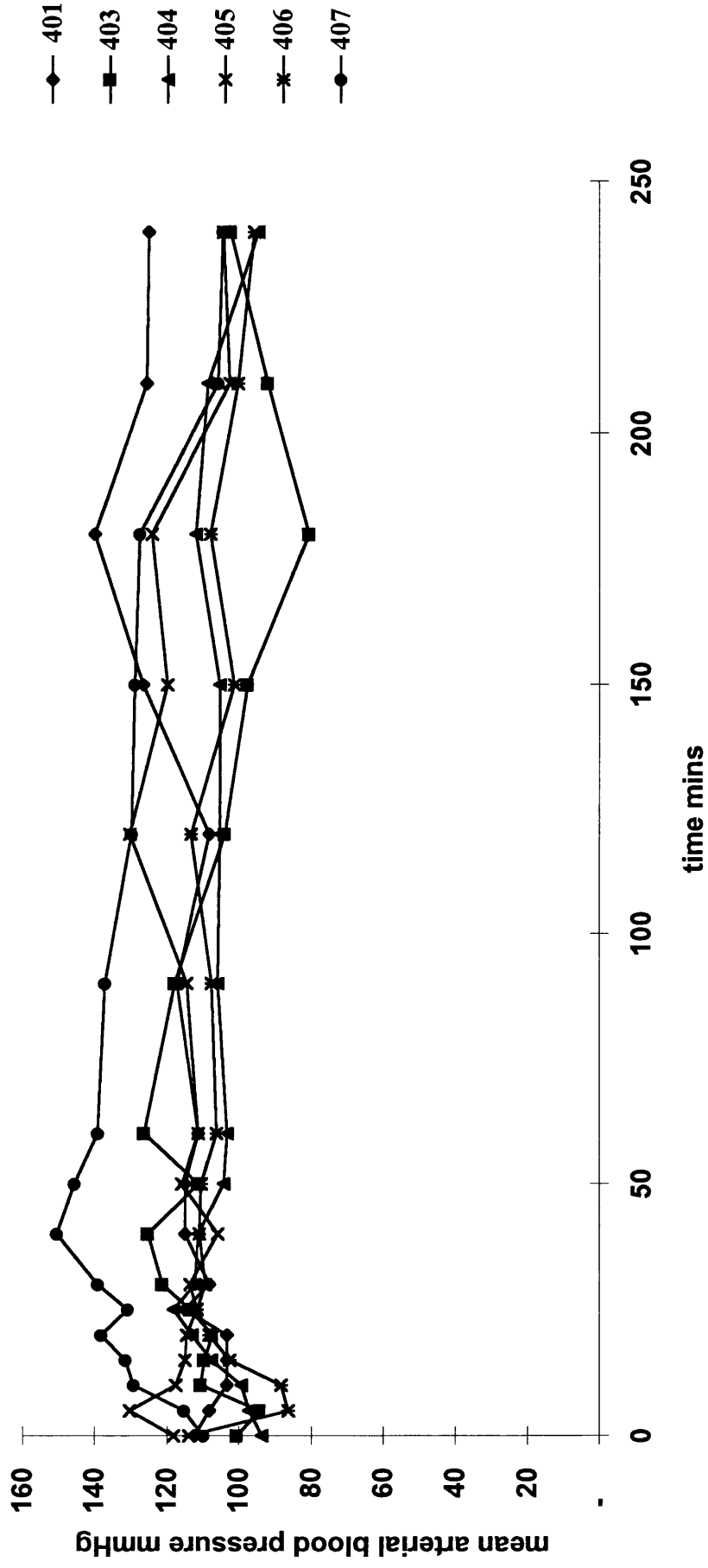


Figure 7.7.3.5 : Mean blood pressure changes (\pm sem) in patients receiving 7.5 mg aptiganel bolus dose.

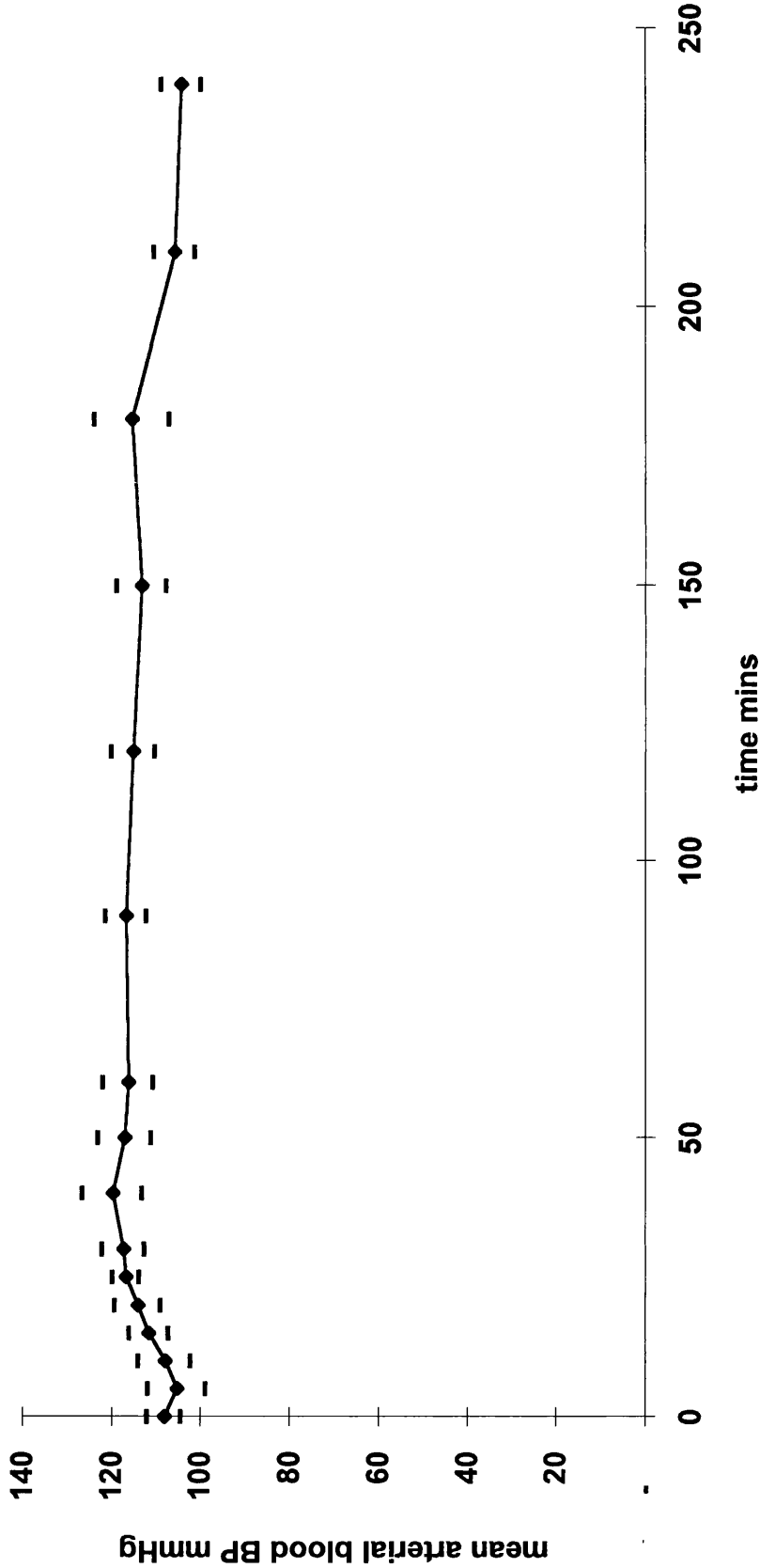


Table 7.7.3.3: CNS adverse events reported in patients receiving bolus aptiganel followed by infusion.

Adverse event	placebo n=5	4.5+0.75mg n=12	6.0+1mg n= 8
headache	1	3	2
visual disturbance	0	3	1
sedation	1	5	10
anxiety	2	5	3
agitation	1	3	4
confusion	0	5	4
hallucinations	0	1	4
insomnia	0	0	1
lightheadedness	1	1	1
total	6/5	26/12	30/8
AE's per patient	1.2	2.2	3.8

Figure 7.7.3.6: Blood pressure vs time in patient 909. Patient received 6+1 mg/hr aptiganel and required intravenous labetolol to control hypertension.

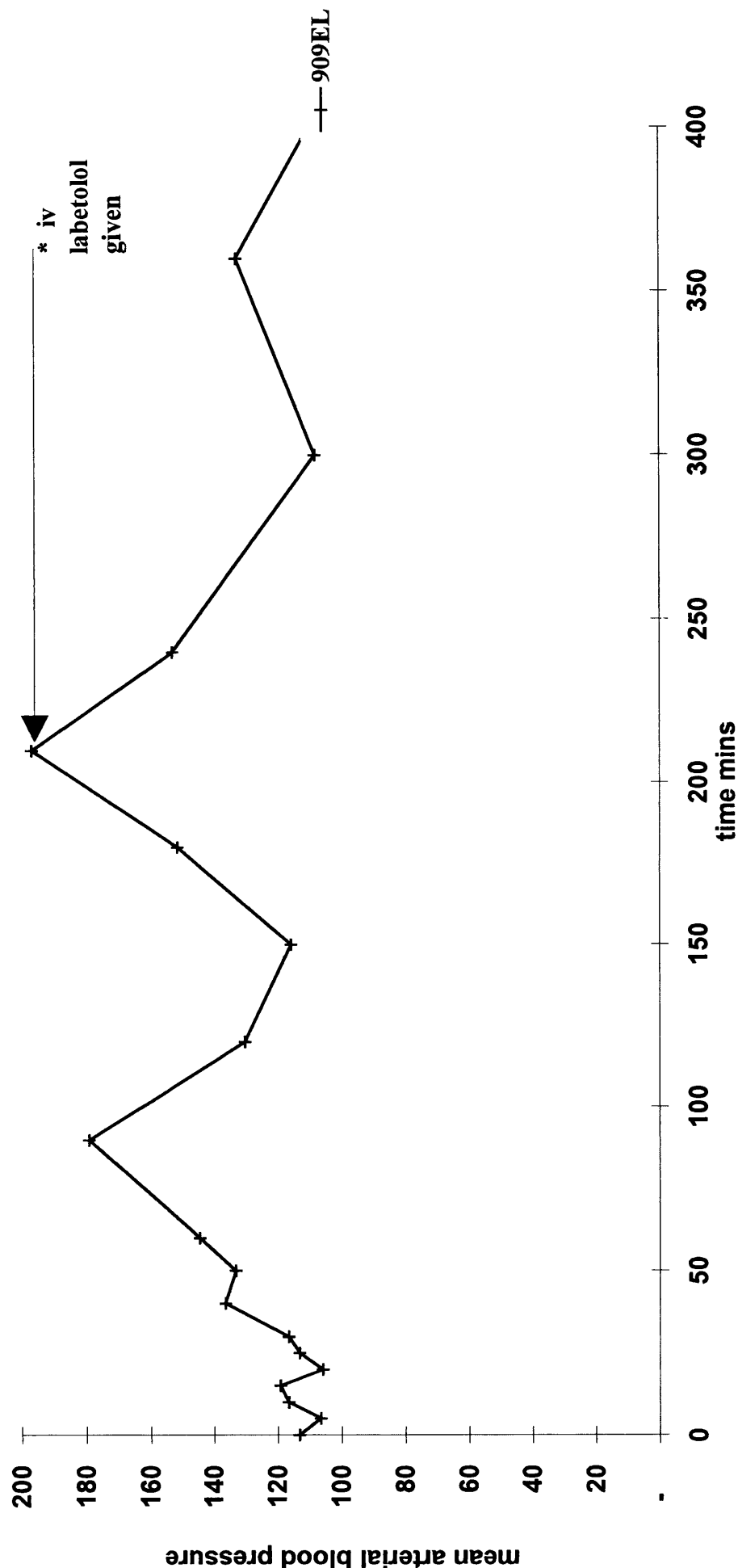


Figure 7.7.3.7: Mean blood pressure vs time (\pm sem) in patients receiving aptiganel 6 mg + 1 mg/h dose

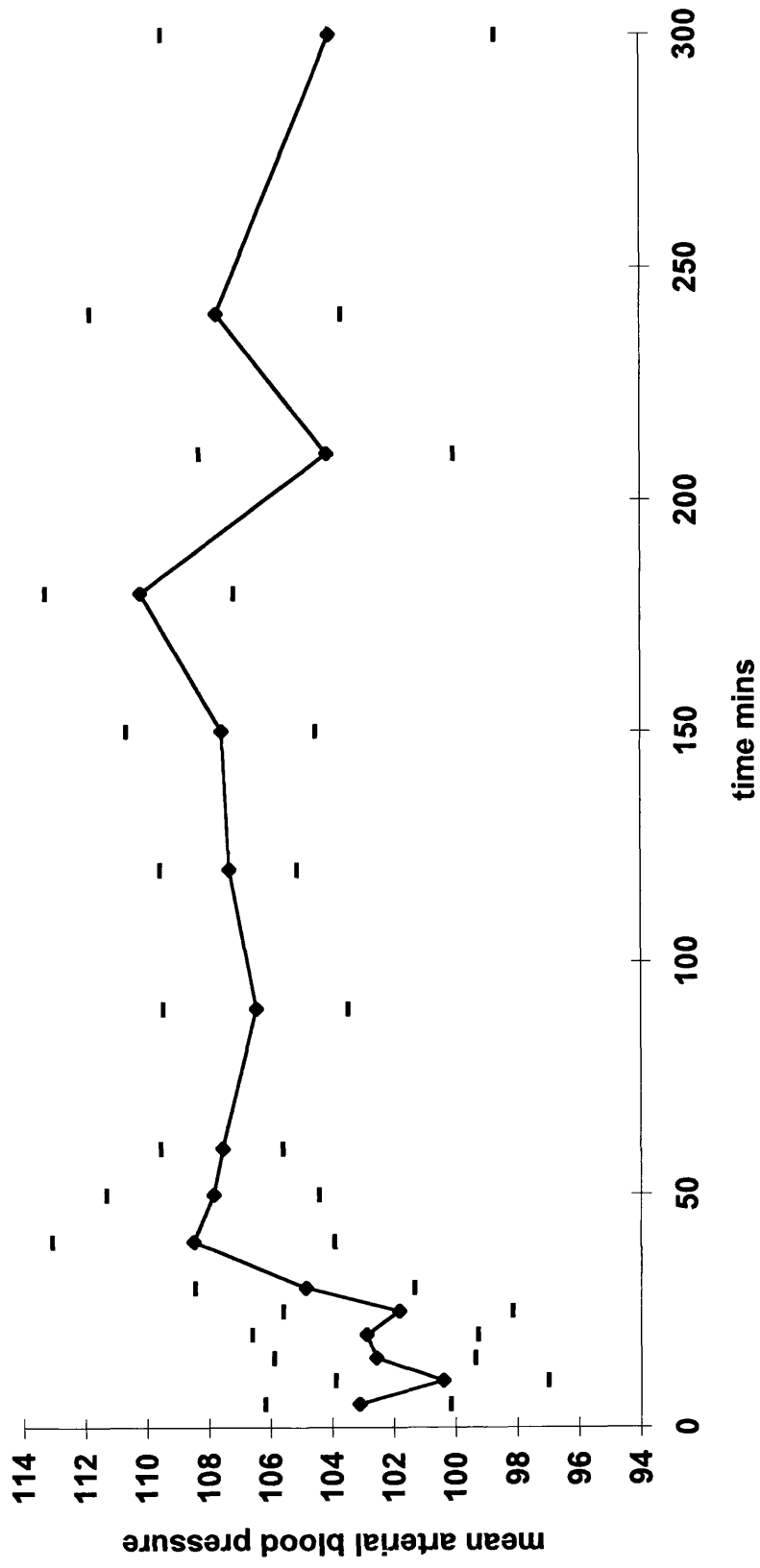


Figure 7.7.3.8: Blood pressure rise in patient 962. Patient received $4.5 + 0.75$ mg/hr aptiganel and required sublingual nifedipine to control hypertension.

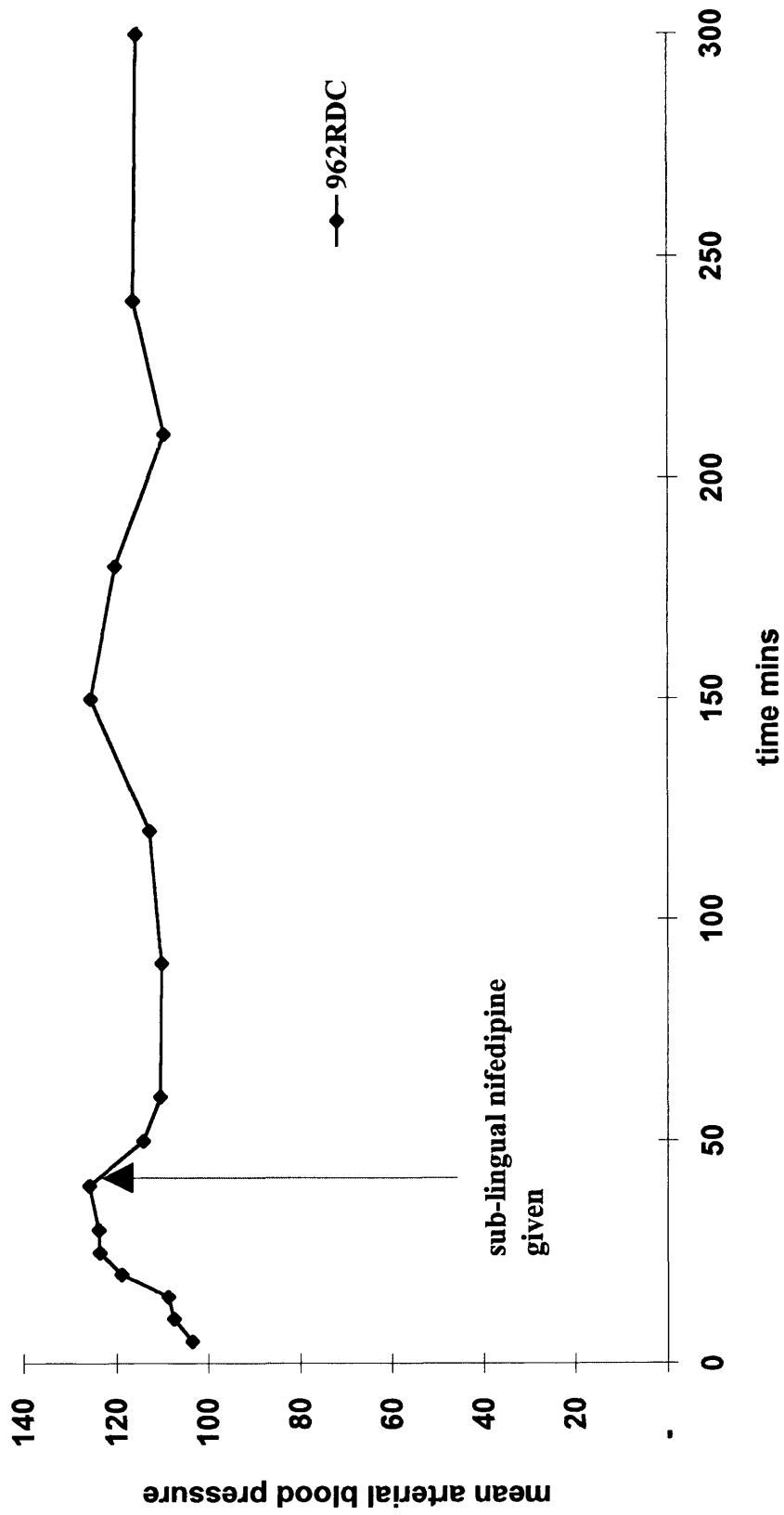


Figure 7.7.3.9: Mean blood pressure vs time (\pm sem) in patients receiving aptiganel 4.5+ 0.75 mg/hr dose

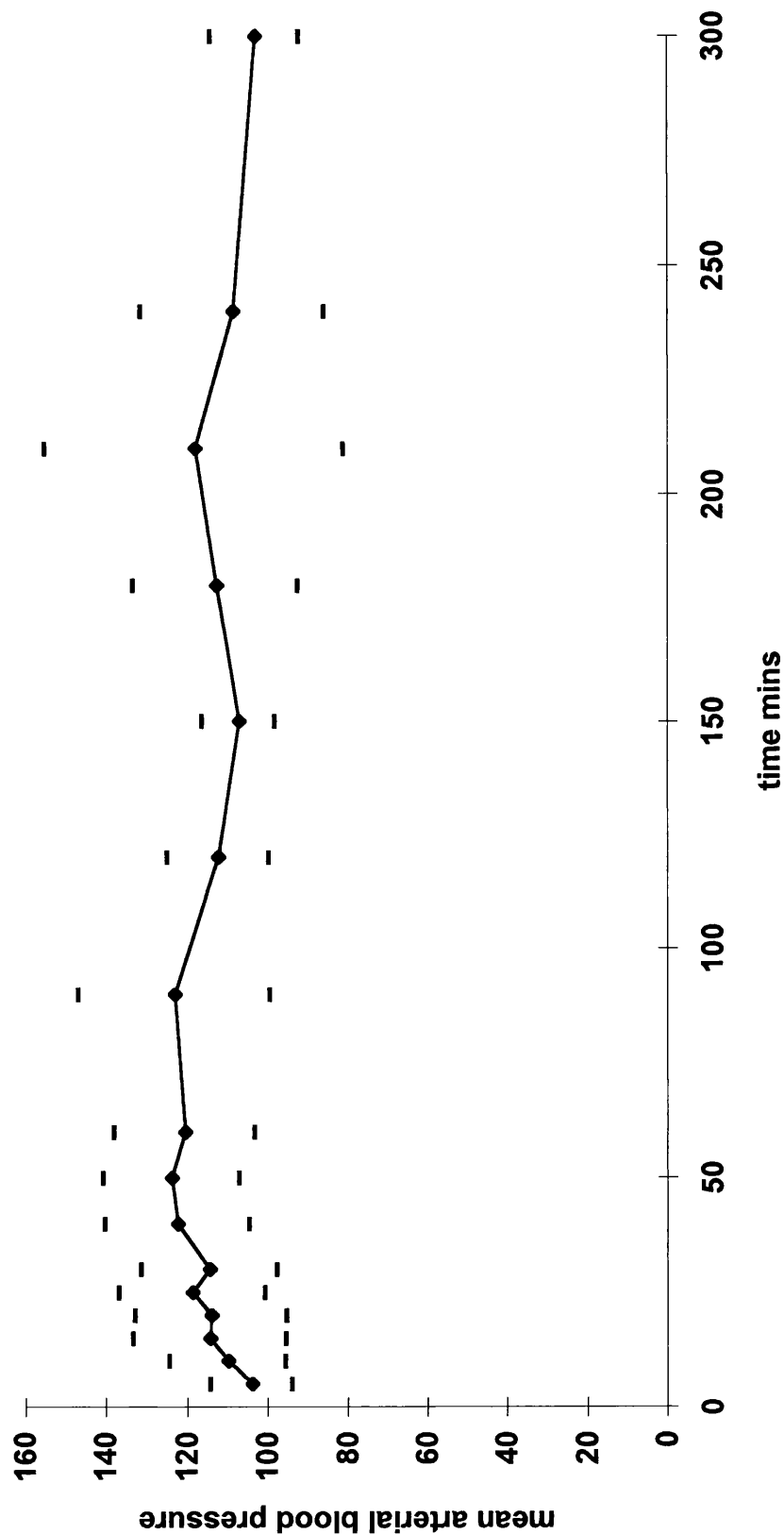


Figure 7.7.6.1: Mean concentrations of aptiganel (\pm sem) vs time. 3mg bolus

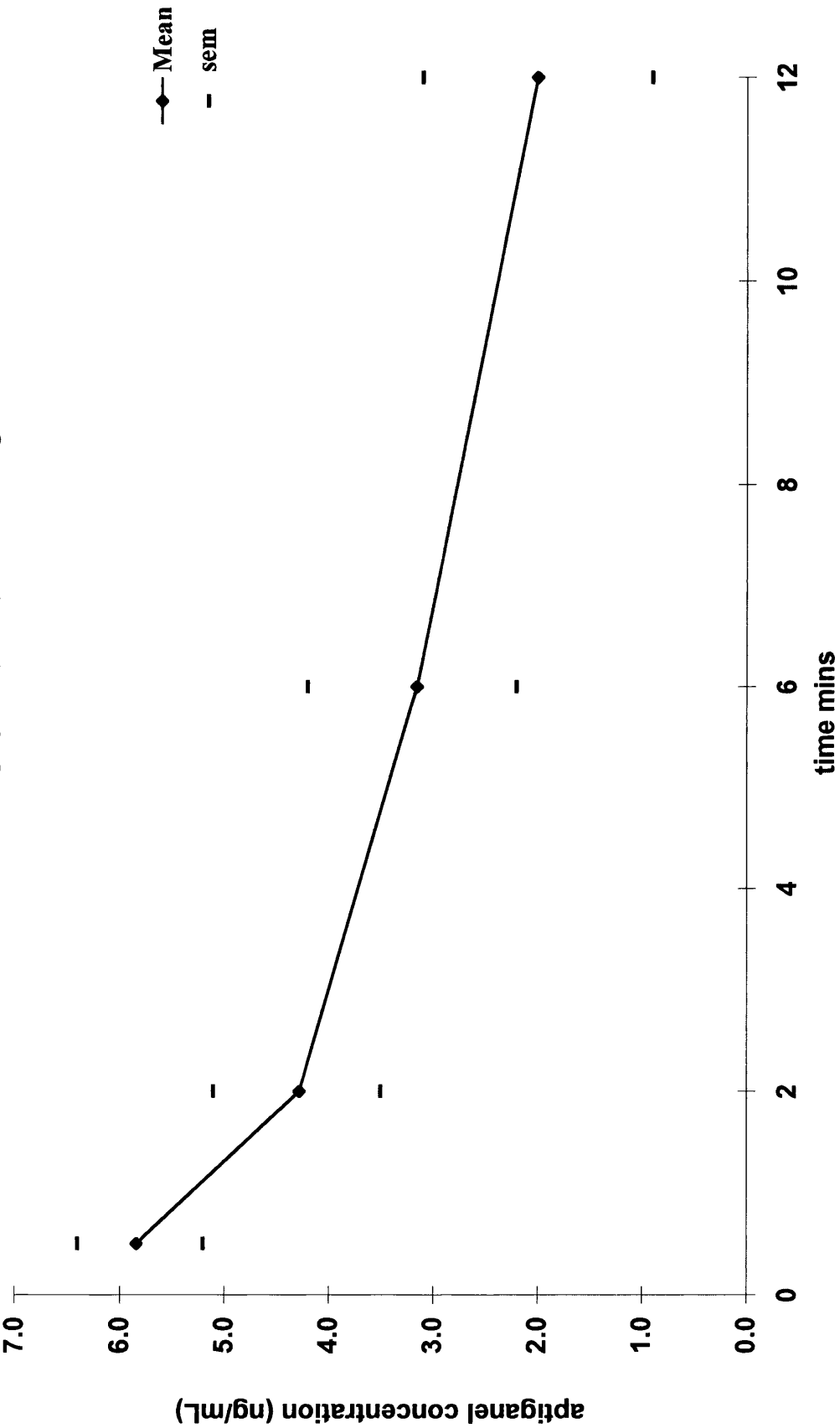


Figure 7.7.6.2: Mean concentration of aptiganel (\pm sem) vs time. 4.5mg bolus

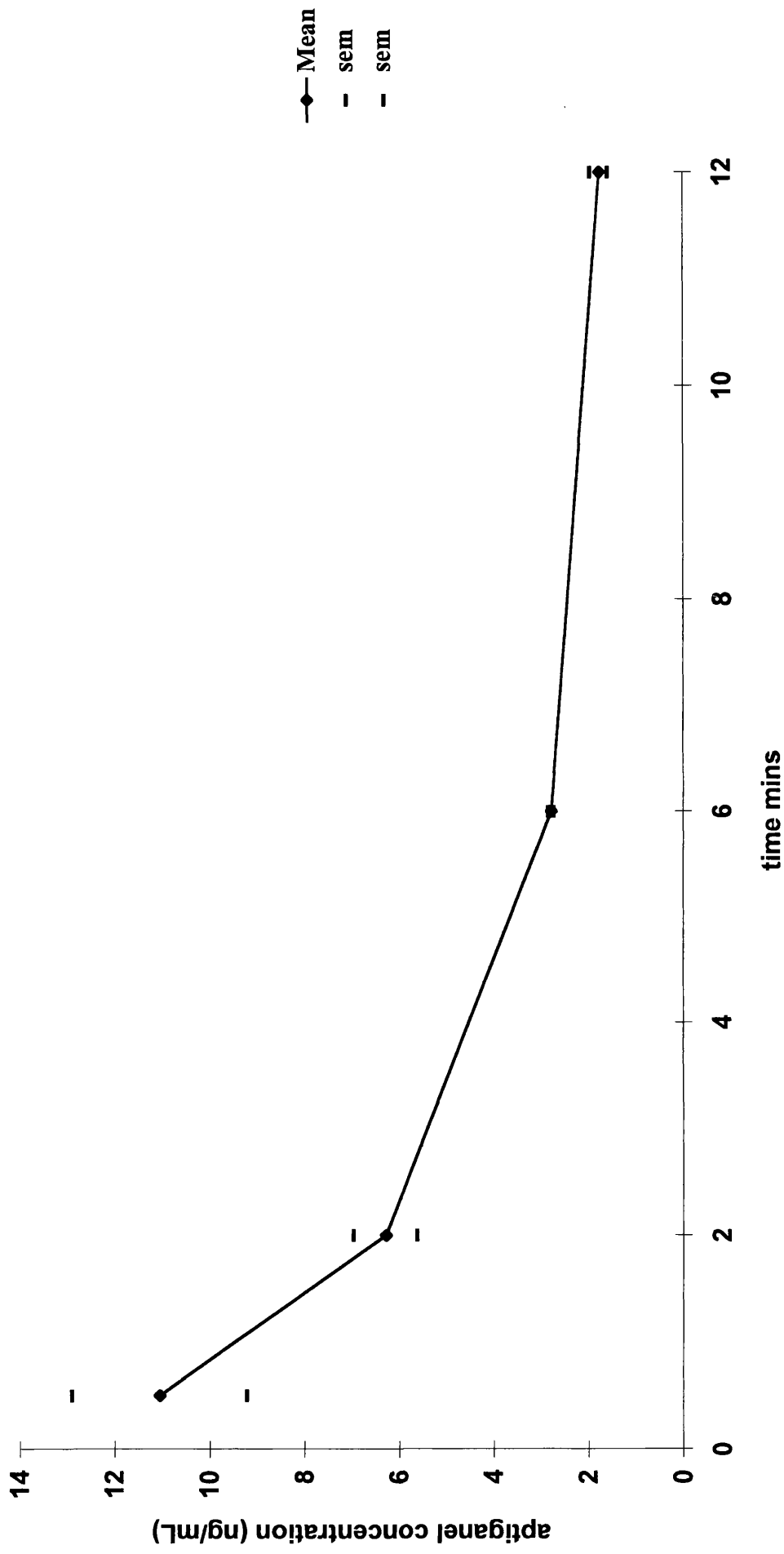


Figure 7.7.6.3: Mean concentrations of aptiganel (\pm sem) vs time. 6mg bolus

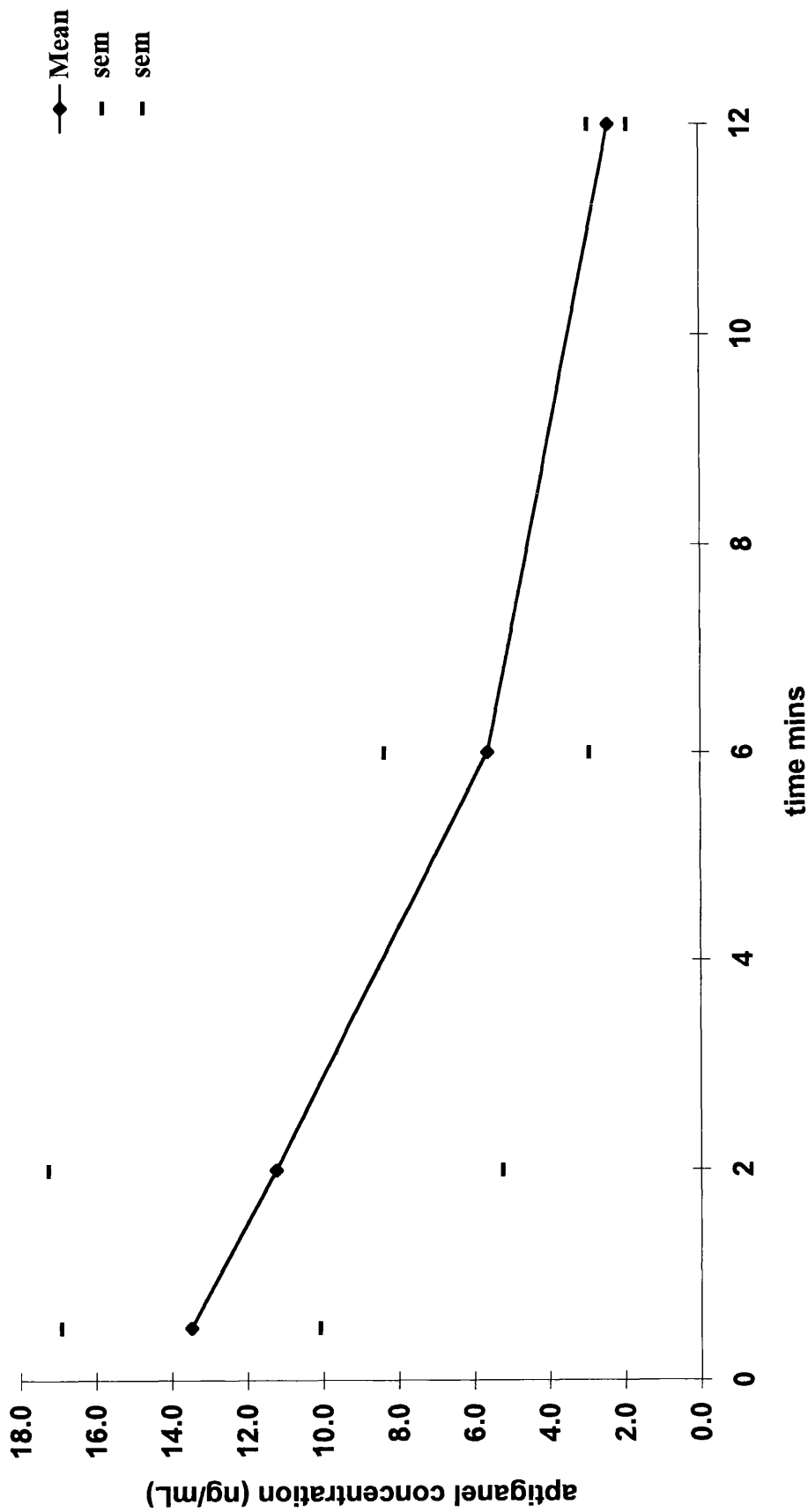


Figure 7.7.6.4: Mean concentration of aptiganel (\pm sem) vs time. 7.5mg bolus

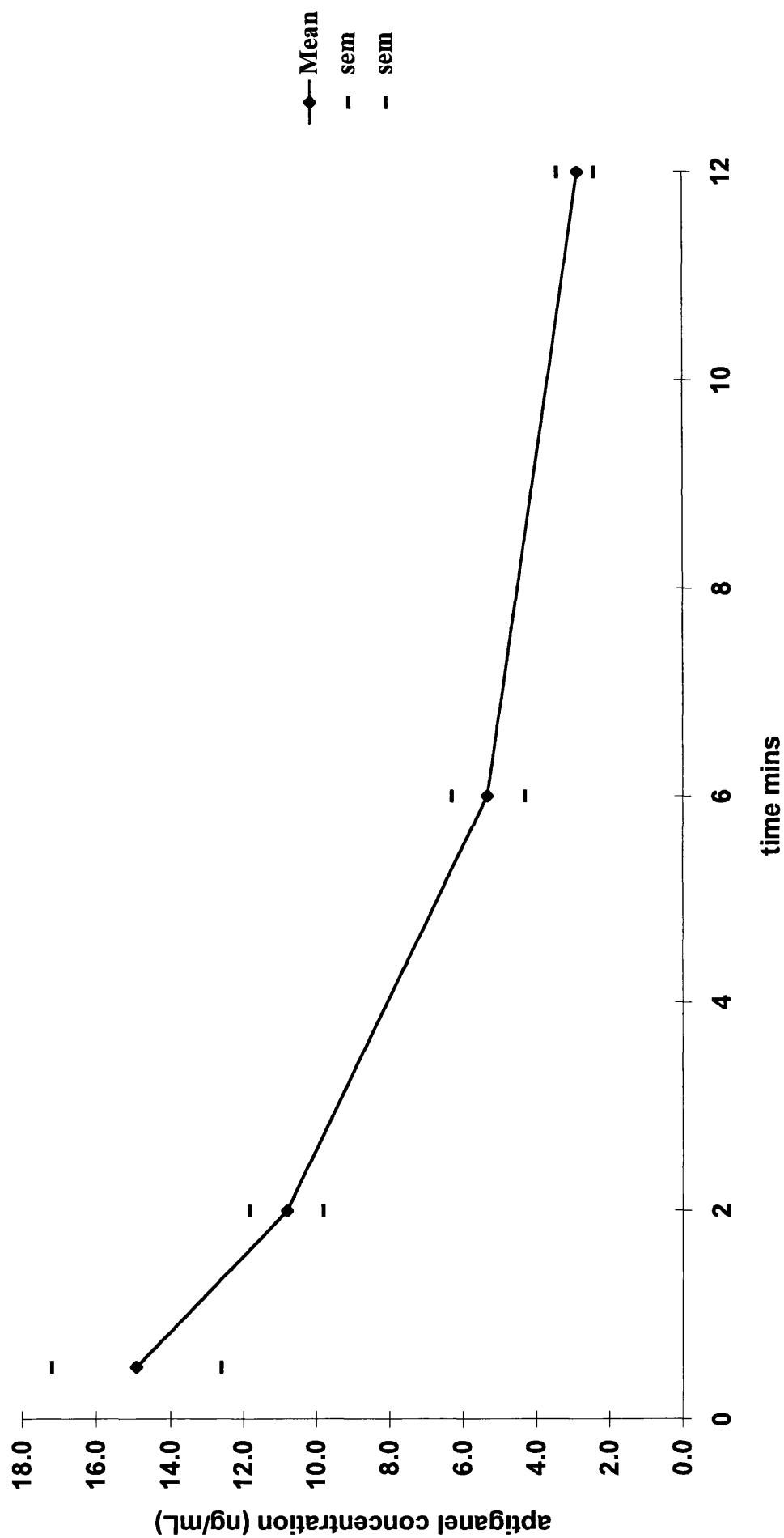


Figure 7.7.6.5: Mean concentrations of aptiganel (\pm sem) vs time. Part B of the study; bolus + infusion

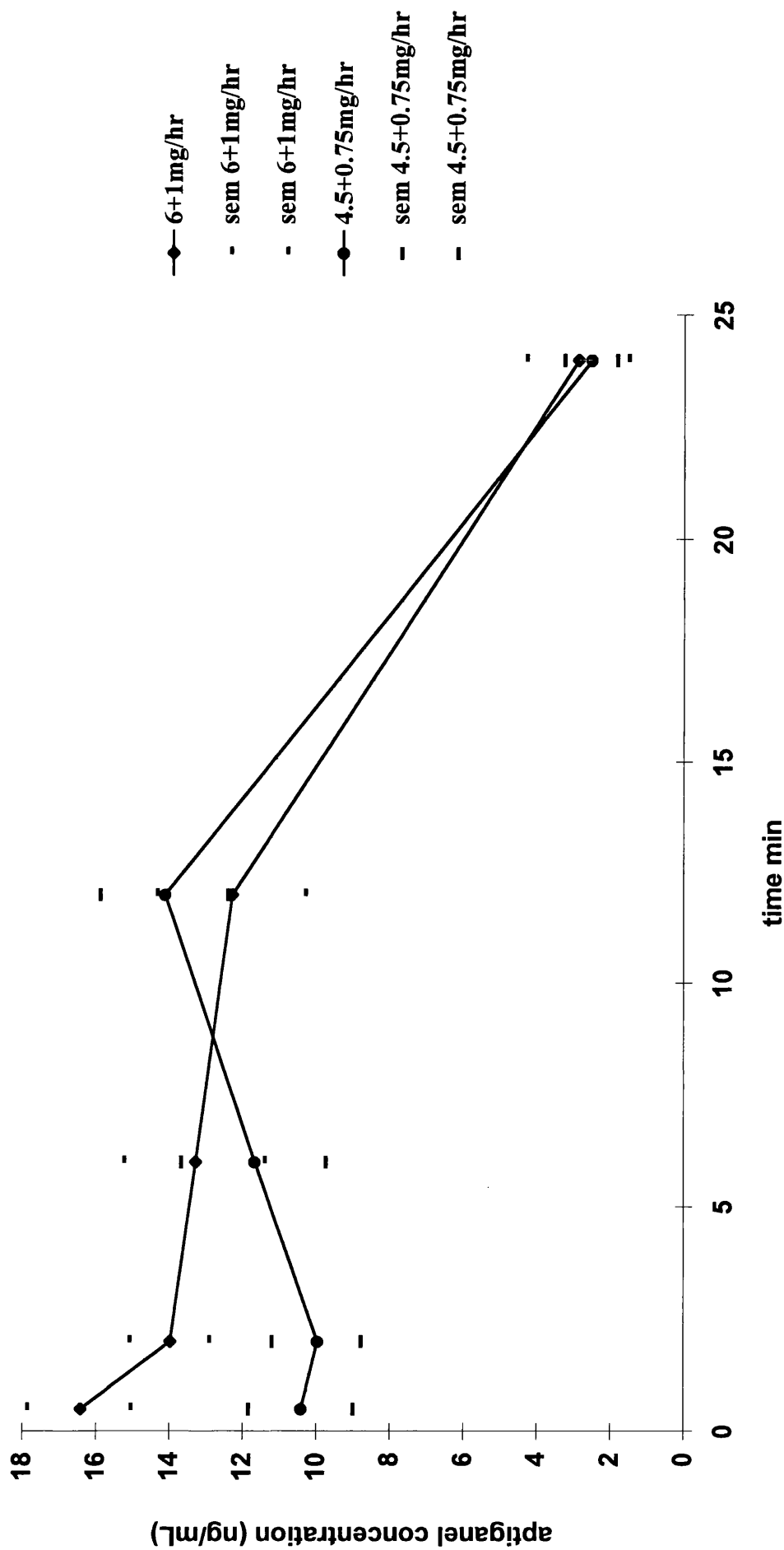


Figure 7.7.7.1: Clinical neurological outcome expressed as NIH Stroke Scale. Graph shows values at presentation and at one month follow up (\pm sem). There was no significant difference in outcome between those patients receiving aptiganel and placebo

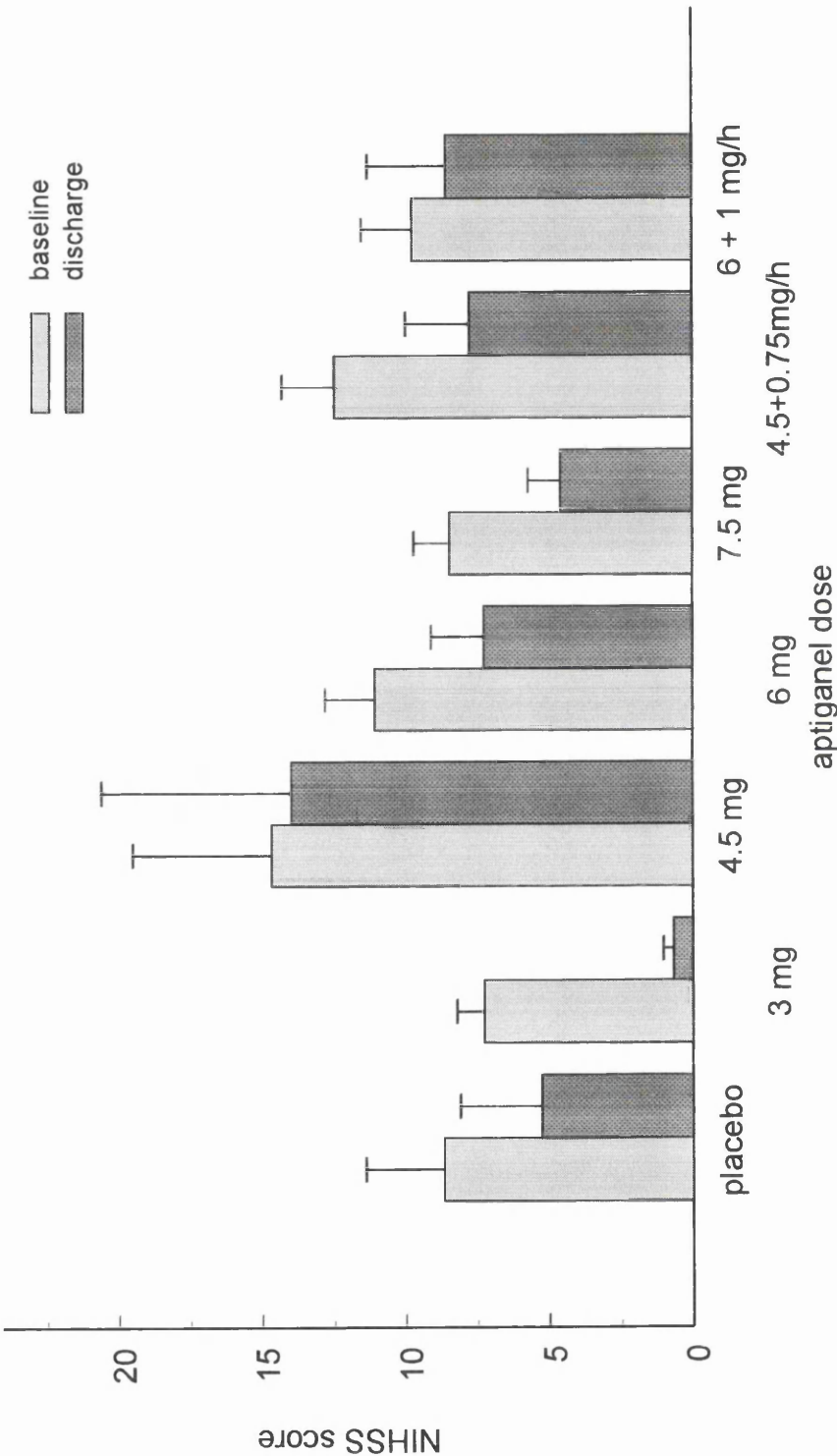
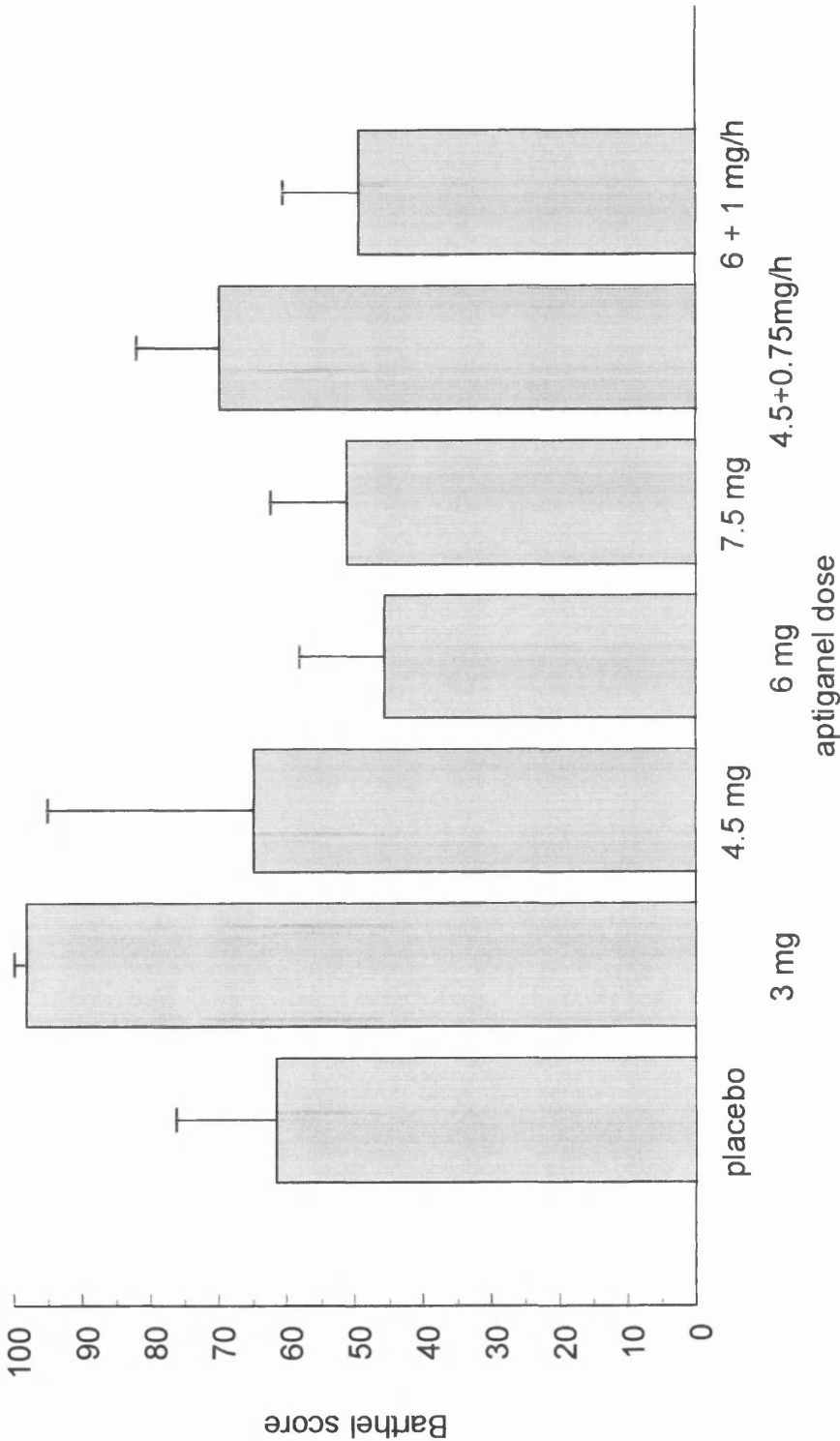


Figure 7.7.7.2: Functional outcome in terms of Barthel score. There was no significant difference in functional outcome comparing patients receiving aptiganel and placebo.



***List of presentations and publications arising from work described in
this thesis***

Chapter 1

Dyker AG, Lees KR, Reid JL. Prevention and management of stroke. In: Hansson L, Birkenhager WH, eds. Handbook of Hypertension: Assessment of Hypertensive Organ Damage, Amsterdam: Elsevier, 1997: 282-302.

Dyker AG, Lees KR. Duration of neuroprotective treatment for ischaemic stroke. **Stroke** (In Press)

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Dyker AG, Grosset DG, Lees KR. Perindopril reduces blood pressure but not cerebral blood flow in patients with recent cerebral ischaemic stroke. **Stroke** 1997; **28**: 580-583.

Dyker AG, Grosset DG, Lees KR. Effect of perindopril on blood pressure and cerebral blood flow after acute ischaemic stroke. **Eur J Neurology** 1995; **2**: 7(Abtract)

Chapter 3

Dyker AG, Weir CJ, Lees KR. Risk factors for stroke in the west of Scotland.

Cerebrovascular Diseases 1996; **6**: 96-P479.(Abstract)

Dyker AG, Weir CJ, Lees KR. Serum cholesterol in patients with acute stroke.

Cerebrovascular Diseases 1996; **6**: 96-P478.(Abstract)

Dyker AG, Weir CJ, Lees KR. Influence of cholesterol on survival after stroke: retrospective study. **British Medical Journal** 1997; **314**: 1584-1588.

Dyker AG, Lees KR. Influence of cholesterol on survival after stroke: retrospective study.

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Weir CJ, Murray GD, Dyker AG, Lees KR. Hyperglycaemia independently predicts survival following acute stroke. **Cerebrovascular Diseases** 1996; **6**: 11-No. 58.(Abstract)

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Dyker AG, Lees KR, Preston C, Caveggion E. The safety, tolerability and pharmacokinetics of GV150526A in patients with acute stroke. **Stroke** 1997; **28**: 233 (Abstract)

Hoke JF, Dyker AG, McAllister AM, Lees KR. Pharmacokinetics of GV150526A following multiple intravenous doses in acute stroke patients. **Cerebrovascular Diseases** 1997; **7(suppl 4)**: 29 (Abstract)

Dyker AG, Lees KR. The safety and tolerability and pharmacokinetics of GV150526A (a novel glycine antagonist) in patients with acute ischaemic stroke. **Proceedings of the Third International Conference on Neuroprotective Agents, Varenna, Italy, 9-12 September 1996**; (Abstract)

Chapter 6

Dyker AG, Muir KW, Lees KR. A double-blind, randomised, placebo controlled, dose-escalation study of remacemide in patients with acute stroke. **Stroke** 1997; **28**: 233(Abstract)

Dyker AG, Muir KW, Lees KR. A double-blind, randomized, placebo-controlled, dose-escalation study of the moderate affinity NMDA antagonist remacemide in patients with acute ischaemic stroke. **Proceedings of the Third International Conference on Neuroprotective Agents, Varenna, Italy, 9-12 September 1996**; (Abstract)

Chapter 7

Fayad P, Edwards K, Holmes J, Lees KR, Dyker AG. The safety and tolerability of non weight-adjusted doses of aptiganel HCl (CERESTAT) in acute ischemic stroke patients. Presented at 21st International Joint Conference on Stroke and Cerebral Circulation, San Antonio, Texas, February 1996 (Abstract)

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Appendix I

NIH Stroke Scale

The NIH Stroke Scale is an established reliable tool for the assessment of patients with acute stroke. It can be reliably performed by trained nursing and medical staff.¹²⁹

NIH Scale	NIH
Level of Consciousness	Score
0 = Alert, keenly responsive	
1 = Drowsy, but rousable by minor stimulation to obey, answer or respond	
2 = Stuporous, requires repeated stimulation to attend, or lethargic or obtunded, requiring strong or painful stimulation to make movements	<input type="checkbox"/>
3 = Coma, responds only with reflex motor or autonomic effects, or unresponsive	
Level of Consciousness - Questions	
Ask patient the month and his/her age. Score first answer.	
0 = Answers both correctly	
1 = Answers one correctly	<input type="checkbox"/>
2 = Incorrect	
Level of Consciousness - Commands	
Ask patient to open/close hand and eyes. Score if he or she makes unequivocal attempt.	
0 = Obeys both correctly	
1 = Obeys one correctly	<input type="checkbox"/>
2 = Incorrect	
Pupillary Response	
0 = Both reactive	
1 = One reactive	<input type="checkbox"/>
2 = Neither reactive	
Best Gaze	
0 = Normal	
1 = Partial gaze palsy, abnormal but not forced deviation	<input type="checkbox"/>
2 = Forced deviation/total gaze paresis	
Best Visual	
Confrontation testing using finger movements, including double simultaneous stimulation. Use visual threat if consciousness or comprehension limit testing, scoring '1' for any asymmetry demonstrated.	
0 = No visual loss	
1 = Partial hemianopia	<input type="checkbox"/>
2 = Complete hemianopia, to within 5 degrees of fixation	

continues

NIH Scale, continued

NIH

Score

Facial Palsy

- 0 = Normal
- 1 = Minor
- 2 = Partial
- 3 = Complete

☐

Best Motor - Arm

Arms held for 10 seconds at 90 degrees if sitting, 45 degrees if lying. Grade weaker arm. Place arms in position if comprehension reduced.

- 0 = No drift in 10 seconds
- 1 = Drift, after brief hold
- 2 = Cannot resist gravity, falling immediately but some effort made
- 3 = No effort against gravity

☐

Best Motor Leg

While lying, patient to hold weaker leg raised 30 degrees for 5 seconds. Place leg if comprehension reduced.

- 0 = No drift in 5 seconds
- 1 = Drift, lowering within 5 seconds
- 2 = Cannot resist gravity, falling to bed but some effort made
- 3 = No effort against gravity

☐

Plantar Reflex

- 0 = Normal
- 1 = Equivocal
- 2 = One extensor
- 3 = Bilateral extensor

☐

Limb Ataxia

Finger-nose and heel-to-shin tests performed; ataxia is scored only if out of proportion to weakness. If total paralysis, score as absent.

- 0 = Absent
- 1 = Present in arm or leg
- 2 = Present in arm and leg

☐

continues

NIH Scale, continued

NIH

Score

Sensory

Tested with pin; only hemisensory loss record. If comprehension or consciousness reduced, only score if obvious evidence.

- 0 = Normal
- 1 = Partial loss, subjectively different but still felt
- 2 = Dense loss, unaware of being touched

Neglect

- 0 = No neglect
- 1 = Partial neglect; visual, tactile or auditory
- 2 = Complete neglect; affecting more than one modality

Dysarthria

- 0 = Normal articulation
- 1 = Mild to moderate dysarthria, slurring some words
- 2 = Near unintelligible or worse

Best Language

Assessed from responses during evaluation.

- 0 = No aphasia
- 1 = Mild to moderate aphasia; naming errors, paraphrasia, etc
- 2 = Severe aphasia
- 3 = Mute

Total Score (0-36) =

Canadian Stroke Scale

1. MENTATION

- Level of Consciousness: Alert = Normal; Drowsy = Awake but dozes;
- Orientation: Orientated = Place + Time
Not Applicable = Comprehension or Speech Deficit
- Speech: Repeat 3 commands (twice each if necessary).
- Receptive Deficit = Obeys 2 or less commands.
If receptive deficit, proceed to testing motor function.
If obeys, ask patient to name 3 objects (pencil, watch, key).
- Expressive Deficit = Names 2 or less objects.
If can name objects, ask "What do you do with a pencil/
watch/key?"
- Normal = Answers 3 questions correctly.
- Expressive Deficit = Answers 2 or less questions correctly.

2A. NO COMPREHENSION DEFICIT - MOTOR FUNCTION

WEAKNESS

- None = No detectable weakness.
- Mild = Normal movement against gravity but succumbs to resistance.
- Significant = Cannot completely overcome gravity.
- Total = Absence of motion or only contraction of muscles without movement of limb.

2B. COMPREHENSION DEFICIT - MOTOR FUNCTION

- Face: Symmetrical = Symmetrical response to pain, eg sternal pressure.
- Arms: Equal = Maintain fixed posture for a few seconds or withdraw equally to pain.
- Legs: Equal = Maintain fixed posture for a few seconds or withdraw equally to pain.

Appendix III
Barthel index

The Barthel is a functional evaluation of recovery following stroke¹²⁹

Barthel Index	BARTHEL
Bowels	Score
0 = Incontinent (or needs to be given enemata)	<input type="checkbox"/>
1 = Occasional accident (once/week)	
2 = Continent	
Bladder	
0 = Incontinent or catheterised and unable to manage alone	<input type="checkbox"/>
1 = Occasional accident (max once/day)	
2 = Continent (for over 7 days)	
Grooming	
0 = Needs help with personal care	<input type="checkbox"/>
1 = Independent face/hair/teeth/shaving (implements provided)	
Toilet Use	
0 = Dependent	<input type="checkbox"/>
1 = Needs some help, but can do something alone	
2 = Independent (on and off, dressing, wiping)	
Feeding	
0 = Unable	<input type="checkbox"/>
1 = Needs some help, but can do something alone	
2 = Independent (food provided in reach)	
Transfer	
0 = Unable - no sitting balance	<input type="checkbox"/>
1 = Major help (one or two people, physical) can sit	
2 = Minor help (verbal or physical)	
3 = Independent	
Mobility	
0 = Immobile	<input type="checkbox"/>
1 = Wheelchair independent, including corners, etc	
2 = Walks with help of one person (verbal or physical)	
3 = Independent (but may use any aid, eg stick)	
Dressing	
0 = Dependent	<input type="checkbox"/>
1 = Needs help but can do about half unaided	
2 = Independent (including buttons, zips, laces, etc)	
Stairs	
0 = Unable	<input type="checkbox"/>
1 = Needs help (verbal, physical, carrying aid)	
2 = Independent up and down	
Bathing	
0 = Dependent	<input type="checkbox"/>
1 = Independent (or in shower)	
Total Score (0 - 20)	<input type="checkbox"/>

